TECHNICAL UNIVERSITY OF LISBON

Faculty of Veterinary Medicine

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DEDICATION

For everyone that influenced my life, especially:

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This dissertation is the most important project during my student career and it couldn't be possible without many people's effort.

First of all, I would like to thank my parent's, my brother and boyfriend who always supported and encouraged me to go further.

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ABSTRACT

Risk assessment for Bluetongue virus vectors occurrence based on geographical Information Systems and statistical modeling

Bluetongue (BTD) is amongst the World Animal Health Organization (OIE) listed diseases due to its potential for rapid spread and serious economic impact on livestock. Because of its epidemiology, in the past, only Southern European countries were affected by the disease. However in the latter half of 2006 an unprecedented outbreak of Bluetongue virus (BTV)-8 occurred in North-western European countries.

To define potential regions that are at risk for BTD epidemics it's essential to study the vector's distribution and abundance. Thus, this study is concerned with the BTD vectors spread, mostly in Austria. The objective is to produce risk maps with the more suitable areas for the occurrence of the vectors, hence to support and conduct the process of preventing and controlling the BTD.

The introductory review gives an overview of the epidemiology of the disease with focus on the vectors, the recent outbreaks in North-western Europe and the importance of statistical modelling and Geographical Information Systems (GIS) in the process of prediction, prevention and control of BTD. The statistical analysis is based mainly on data from Austrian entomological surveillance system, weather stations and topographical information. A multiple linear regression model was fitted to the data to predict the occurrence of the BTV vectors and subsequently to create the risk maps for the whole country.

Despite the fact that the limited nature of the data does not allow precise estimation, in general, the models indicated that each vector has preferential areas to occur where they can be very abundant.

A more detailed analysis should be carried out with a multidisciplinary team including epidemiologists, biologists, meteorologists, entomologists, statisticians, among others. In this way the complexity of BTD epidemiology might be better understood, resulting in a more efficient process of prevention and control of this disease.

Keywords*:* BTD, vectors, surveillance, risk, GIS and modelling.

RESUMO

Análise do risco da ocorrência de vectores da Língua Azul com base em Sistemas de Informação Geográfica e modelos estatísticos.

A Língua Azul (LA) está entre as doenças da lista da Organização Mundial de Saúde Animal (OIE) devido ao seu potencial de rápida disseminação e do grave impacto económico na pecuária. No passado, devido à sua epidemiologia, apenas os países do sul da Europa eram afectados pela doença. No entanto, no segundo semestre de 2006, um surto sem precedentes, de infecção pelo vírus da Língua Azul (BTV) serotipo 8, ocorreu em países do norte da Europa Ocidental.

Para definir as regiões em potencial risco de epidemias de LA é essencial o estudo da distribuição e abundância do vector. O presente estudo baseia-se assim na dispersão de vectores de febre catarral ovina na Áustria. O objectivo é a criação de mapas de risco com as áreas de preferência para esses vectores, no sentido de apoiar e conduzir o processo de prevenção e controle da doença.

A revisão bibliográfica introdutória, contém uma visão geral sobre a epidemiologia da doença com especial destaque para os vectores, para os recentes surtos no Norte da Europa Ocidental e para a importância da aplicação de modelos estatísticos e de Sistemas de Informação Geográfica (SIG) no processo de previsão, prevenção e controle da LA. A análise estatística baseia-se essencialmente nos dados do sistema de vigilância entomológica Austríaco, estações meteorológicas e da topografia do país. Os dados foram analisados com um modelo de regressão linear múltipla com o objectivo de prever a abundância dos vectores da LA e subsequentemente criar os mapas de risco para todo o país.

Apesar do carácter limitado dos dados não permitir uma estimativa precisa, em geral, os modelos indicaram que os vectores possuem áreas preferenciais nas quais podem atingir grande número.

Uma análise mais detalhada deve ser realizada por uma equipe multidisciplinar, incluindo epidemiologistas, biólogos, meteorologistas, entomologistas, estatísticos, entre outros. Desta forma, a complexidade da epidemiologia da LA poderá ser mais facilmente compreendida resultando numa maior eficiência na prevenção e controle da doença.

Palavras-chave: LA, vectores, vigilância, risco, SIG e modelos.

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ABBREVIATIONS

AGES	Austrian Agency for Health and Food Safety
AHSV	African horse sickness virus
AKAV	Akabane virus
BIC	Bayesian information criteria
BTD	Bluetongue
BTV	Bluetongue virus
BTV-8	Bluetongue virus serotype 8
BTV+	Bluetongue virus positive
CGIAR-CSI	Consultative Group for International Agriculture Research - Consortium for Spatial Information
C.	*Culicoides*
DE	Digital elevation
DEM	Digital Elevation Model
EC	European Commission
EEV	Equine encephalitis virus
EFSA	European Food Safety Authority
EHDV	Epizootic haemorrhagic disease virus
EIP	Extrinsic incubation period
ESRI	Environmental Systems Research Institute
EU	European Union
FMV	Faculdade de Medicina Veterinária
GIS	Geographical Information Systems
IAH	Institute for Animal Health
LST	Land Surface temperature
MHFY	Ministry of Health, Family and Youth
MIR	Middle infra-red reflectance
MS	Member State
NDVI	Normalized Difference Vegetation Index
NOAA-AVHRR	National Oceanic and Atmospheric Administration - Advanced Very High Resolution Radiometer
OIE	World Animal Health Organization
PCR	Polymerase chain reaction

QQ	Quantile plots
RH	Relative humidity
spp	The specific names are not specified
ss	*sensu stricto*
TAIR	Temperature of the air
UTL	Universidade Técnica de Lisboa
WAHID	World Animal Health Information Database
ZAMG	Central Institute for Meteorology and Geodynamics

1 TRAINING DESCRIPTION

First part: Portugal

The first part of my training was developed between the 1st October and the 20th of February 2008/2009, in the unit of Epidemiology/Risk Analysis in the Faculty of Veterinary Medicine (FMV) in the Technical University of Lisbon (UTL). During this stage the team, constituted by Dr. Telmo Nunes, Dr. Filipa Baptista and Eng. Hugo Martins introduced me to the following contents:

- epidemiology;
- statistics: including the statistical analysis software SAS®;
- risk analysis;
- Geographical Information Systems (GIS): including ArcGis® and gvSIG.

All of these subjects were learned with theoretical presentations followed by practical exercises.

Second part: Austria

Between the 1st of March and the 28th of May, I worked at the Austrian Agency for Health and Food Safety (AGES) in Graz under Prof. Dr. Klemens Fuchs supervision. This agency includes several departments with various areas of expertise including food inspection, bacteriology and serology, veterinary medicine and, under Prof. Dr. Klemens Fuchs responsibility, data collection, statistics and risk assessment.

The main goal of the ERASMUS experience was to explore a subject, in a useful way for the agency, and at the same time to achieve knowledge about new areas of interest in a different country, thus, with different perspectives and methodologies.

During this period I did several activities including:

- meetings with Prof. Dr Klemens Fuchs (supervisor/ statistician), Dr Johannes Hofrichter (statistician), Dr Angelika Loitsch (veterinary of AGES at Mödling) and Peter Wagner (Chief Veterinary Officer from Styria) to choose the theme of the dissertation and how to explore the data available;
- selecting the data and its sources;
- gather the data;
- data processing;
- statistical analysis including statistical modelling.

I also attended the following courses:

- "Introduction to R" from 2nd to 12th March, by Dr. Johannes Hofrichter;
- "Introduction to Geographical Information Systems I", on 13th March, by M.S. Michael Schwarz;
- "Introduction to Geographical Information Systems II- practice with gvSIG" on 13th March, by MS Michael Schwarz;
- "Biostatistics I - Descriptive Statistics", on 17th April, by Prof. Dr. Klemens Fuchs;
- "Biostatistics I - Inference Statistics", on 8th May, by Prof. Dr. Klemens Fuchs.

2 INTRODUCTION

The Bluetongue disease (BTD) is an arthropod-borne viral disease transmitted by different species of *Culicoides* insects in a range of ecosystems (Wittmann, Mellor & Baylis, 2001).

Because of its economical impact on livestock, BTD is included by The World Organisation for Animal Health (OIE) in the list of immediate reporting diseases and the World Animal Health Information Database - WAHID (World Organisation for Animal Health [OIE], 2008a). This means it has the potential for very serious and rapid spread, irrespective of national borders, and is capable of serious socio-economic consequences that are of major importance in the international trade of animals and animal products (Tatem et al., 2003).

Due to the important role of the vector in Bluetongue Virus (BTV) epidemics, the vast majority of the studies on BTD epidemiology incorporate its capture and identification for further knowledge on its competence, abundance and distribution (Wittmann et al., 2001). This study assess the risk of BTD considering that the entrance, maintenance and abundance of the vector may provide a measure of disease risk before the outbreaks and also target control measures in periods of peak vector activity.

BTD is endemic in the African continent and some areas of Southern Europe, especially in the Mediterranean basin. However, in 2006, while multiple outbreaks of the BTV continued to occur in Southern Europe, unprecedented outbreaks of the BTV serotype 8 (BTV-8) affected five countries in North-Western Europe (European Food Safety Authority [EFSA], 2007a). BTD, which up to then was thought to be limited to regions between latitudes 35° South and 40° North, suddenly appeared at latitudes of more than 50° North (Belgium, Germany, the Netherlands, France and Luxembourg) and has presently affected most European countries (OIE, 2002). Due to its geographical position, Austria, mainly the provinces bordering German areas already affected, was seriously under the threat of a new outbreak.

In June, 2007, Austrian Ministry of Health, Family and Youth (MHFY), aware of BTD spread and impact, initiated the first entomological surveillance programme in the country (Sehnal, Schweige, Schindler, Anderle & Schneemann, 2008). The findings from this program provide a basis for the understanding of *Culicoides* (*C.*) temporal distribution and abundance.

The present work draws from the awareness of the impact of several parameters in vectors ecology, which cannot be discarded, and the consideration of important suggestions concerning BTD ecology as follows.

According with Meiswinkel and his colleagues (2008a) it is now known that the *Culicoides* fauna, endemic to Northern Europe, harbours multiple vectors of BTV. *Culicoides* transmit a

series of globally important pathogens besides BTV and are doing so across an ever-widening geographic range, so there is an urgent and increasing need to identify species with absolute precision.

To enter, spread and establish in a new area, a midge must be active. Flight activity is influenced by a series of edaphic, topographic and climatic factor like temperature, luminosity, humidity and wind speed, amongst others. The length of the period of flight activity is determined by the interrelationships of these factors (EFSA, 2007c). Even though all *Culicoides* species share the same basic habitat requirements, i.e. presence of host for blood-meals and breeding sites for egg and larval development, they differ on their life-history characteristics, and in turn, the extent to which their distribution and abundance is affected by environmental factors (Mellor, Boorman & Baylis, 2000).

Predictive risk maps for BTD derived entirely from distribution data for one species exclude extensive regions at risk of transmission via novel vector species. A full examination of the relative role of different vectors and their climatic requirements, demands the development of models of species abundance rather than presence, based on data collected regularly throughout outbreak periods. The distribution and abundance of these species should be re-modelled using surveillance data from current European Union (EU) project to a local and national level (Purse et al., 2004b). The major issues that have impeded efforts to reduce the impact of BTV on animal health and the economics of animal industries continue to be the accurate prediction of regions with high risk for BTV transmission to livestock; and, within these regions, the prediction of temporal periods of risk before a BTD outbreak. The analysis of spatial information with GIS techniques may identify high risk areas for BTD, in which might be possible, with proper prevention and control measure, to avoid that it becomes endemic (Tabachnick, 2004).

The main goal of this dissertation is to find areas that are more suitable for the main BTV vectors in Austria. According with the results of the entomological program, Obsoletus and Pulicaris complexes are the main vectors captured in the country. The combination of this information with statistical modelling and GIS techniques allowed the creation of risk maps which can help to predict BTV vectors occurrence and abundance. Aiming to improve disease surveillance and control methods, risk maps might give precious help by providing a basis for targeting of financial and monitoring efforts to pre-defined areas.

On the “Review” (chapter 3) there is a brief overview over BTD epidemiology with a special concern about its vectors (3.1.3). On chapter 3.2 the BTD history is developed including the outbreak in North-western Europe (3.2.1), with the Austrian situation in detail (3.2.3). BTD prevention and control comes on chapter (3.4) specially focusing on surveillance strategies

(3.4.1). The risk factors for *Culicoides* come in a separated chapter (3.5), considering its importance to understand the introduction, establishment and spread of BTV vectors. In the end of the review there is a brief explanation about the modelling process (3.6) and GIS technologies (3.7) to achieve the risk maps (3.8), which will be fully explained later in the "Material and methods" (4). In the previous chapter an explanation of the whole building process of this work is presented starting with the selection of the data sources (4.2.1), collection (4.2.2) and processing (4.3); followed by the statistical modelling (4.4) to achieve the risk maps (4.5). The "Results" (5) and "Discussion" (6) follow the same order described before. The conclusions are presented on chapter 7.

3 REVIEW

3.1 **BTD epidemiology**

BTD is a vector borne viral infection transmitted to ruminants by species of *Culicoides* insects.

3.1.1 **Virus**

BTV is an *Orbivirus* belonging to the family *Reoviridae* with 70 µm size and 24 known serotypes (Mehlhorn et al., 2007).

The distribution of the BTV has been described by Tabachnick, Mellor & Standfast (1992) and is limited to geographic areas with competent vector species. Vectors of BTV elsewhere in the world are known to transmit also other viral pathogens, such as African horse sickness virus (AHSV), Akabane virus (AKAV), Epizootic haemorrhagic disease virus (EHDV) and Equine encephalitis virus (EEV) (Mellor & Leake, 2000).

3.1.2 **Hosts and symptoms**

According with Barnard (1997) all ruminants are probably susceptible to BTV infection. BTD affects a wide range of species including: cattle, sheep, goats, dromedaries and wild ruminants. However the incidence of clinical disease varies by serotype, strain and host specie (Wilson & Mellor, 2008).

BTD can induce in sheep cyanotic blue tongue due to oedema in the pharyngeal cavity and food aspiration into the respiratory tract. After a mean incubation period of 6-9 days the symptoms described include fever with approximately 41 ºC, severe nasal discharge, lacrimation, facial oedema and nasal excoriations. Typical lesions appear involving coronitis and inflammation of the whole claw region. Foot is hot to touch and movements of the animal are stiff. The lymph nodes are swollen, oedematous and hemorrhagic. In the whole mouth appear ulcers and haemorrhagic lesions complicated with bacterial infections. There are also oedemas at the udder, in the lung and trachea. In severe cases asphyxiation might occur and also leucopenia, hyperaemia, lethargy, anorexia, torticollis and possibly death (Mehlhorn et al., 2007).

In cattle, the disease is usually subclinical, although clinical signs appear to occur often during the initial introduction of BTV into a naïve population (Parsonson, 1990). Even subclinical infection may carry significant associated costs, including loss of body condition, reduced milk yield, infertility and abortion (Osburn, 1994), while indirect costs are largely

created by the export restrictions and surveillance requirements imposed to limit the spread of the virus (Hoogendam, 2007).

Culicoides are opportunistic also feeding upon birds and mammals, but they are strongly attracted to large mammals. The importance of the role of wildlife species remains to be assessed. Studies in North America suggest that wild ruminants may play a part in BTD epidemiology depending upon the size of the populations and their levels of contact with local domestic livestock. The part played by other wildlife species (e.g. carnivores) in BTV transmission cycle, also remains to be assessed (EFSA, 2007b). Nevertheless the vast majority feed upon cattle and sheep varying the body region from host to host (EFSA, 2008a).

3.1.2.1 Viraemia

Ruminants naturally infected with one serotype of BTV have a solid, lifelong immunity to the homologous serotype, but only partial or no protection against other (heterologous) serotypes. This prolonged blood infection of ruminants increases the likelihood that feeding insect vectors will acquire infection, and prolonged infection might also complicate the safe movement of animals between BTV-free and infected regions. The incubation period following natural BTV infection of sheep is estimated at 7 days, but can range between 2-15 days. In sheep the viraemia rarely persists for longer than 14 days and is usually present for 6 to 8 days. In cattle is typically prolonged to about 50 days (Verwoerd and Erasmus, 2004).

3.1.2.2 Transmission between hosts

BTD is not contagious and very little virus are found in the secretions and excretions of infected animals. One exception is semen from viraemic bulls which can infect cows (Bowen, Howard & Pickett, 1985). Transmission of BTV from dam to progeny can occur by transplacental transfer and by oral infection of neonates by infected milk or colostrums (EFSA, 2008a), but their role, together with embryo transfer, seems to be negligible.

3.1.3 *Culicoides*

Ceratopogonidae are small biting midges (Insecta: Diptera) comprising about 6000 species in 110 genera (Mullen, 2009). In four genera, including *Culicoides* there are hematophagous females (Kampen & Kiel, 2006). Within *Culicoides*, 96% are obligatory blood suckers in mammals (Mellor et al., 2000). Fifty species are known to act as vectors for human and animal diseases, approximately 30 *Culicoides* species act as vectors for BTV worldwide. Adult females of *Culicoides* species are the main vectors. According with Mehlhorn and his colleagues (2007), seven species were proven as true vectors while, in experiments, 17 other species were found to be suitable hosts.

The subgenus *Avaritia* include the Obsoletus complex, the *C. chiopterus*, *C. dewulfi* and *C. imicola* (EFSA, 2008a), whereas *C. pulicaris*, *C. lupicaris* and *C. punctatus* belong to the subgenus *Culicoides* (Meiswinkel et al., 2008b).

3.1.3.1 Distribution and sympatry

BTV vectors are distributed across the globe from the tropics to the tundra and from sea level to 200 m (in Tibet). Approximately 120 species of *Culicoides* occur in Europe; this diversity declines markedly towards the Arctic, with only about 25 species living beyond the 60º latitude.

In the past, *C. imicola* was by far the most important BTV vector specie. In Europe, *C. imicola*, *C. obsoletus*, *C. scoticus*, *C. dewulfi*, *C. pulicaris* and, very recently, *C. chiopterus* have been implicated in BTV transmission (Meiswinkel, van Rijn & Lejis, 2007). In fact, in all of the area without *C. imicola* where BTV has been detected, *Culicoide*s of the Obsoletus and Pulicaris groups are by far the most abundant and prevalent biting-midge species (Mellor & Pitzolis, 1979).

Culicoides obsoletus and *C. scoticus*, with four other species that occur further eastwards across the Holarctic region, comprise the Obsoletus complex (EFSA, 2007c). Although they are believed responsible for only 10% of BTV transmission in Europe it appears that they are beginning to play an increasingly more important vectorial role. The first findings to positively incriminate *C. obsoletus* midge as BTV vectors occurred in southern Europe on the island of Cyprus (Mellor & Pitzolis, 1979). Its status as a vector was reaffirmed in 2003, together with its related *C. scoticus* (EFSA, 2007c). There, while *C. imicola* is scarce or absent, Obsoletus Complex midges apparently comprised more than 90% of over 10 000 collected *Culicoides* (Savini, Goffredo, Monaco, de Santis & Meiswinkel, 2003).

Most authors equal *Culicoides* subgenus with *C. pulicaris* group or the Pulicaris complex. More correctly, Pulicaris complex comprises only 12 species of which, at least, eight are found in Europe (EFSA, 2007c). Each taxon within the Pulicaris complex inhabits an almost exclusive biotope which will influence greatly its distribution, both in space and time. During the outbreak of BTV-8 in North-Western Europe (2006) *C. pulicaris sensu stricto* (*ss*) was found to comprise less than 5% of approximately 100 000 *Culicoides* captured and was also limited in its distribution (EFSA, 2007c). *C. pulicaris ss* was first incriminated as BTV vector in 2003, on the island of Sicily, when Caracappa and his colleagues made five isolations of BTV in midges from this specie, at locations and times when *C. imicola* was absent. These are the first field isolations ever reported from this species. Still, in Northern Europe *C.*

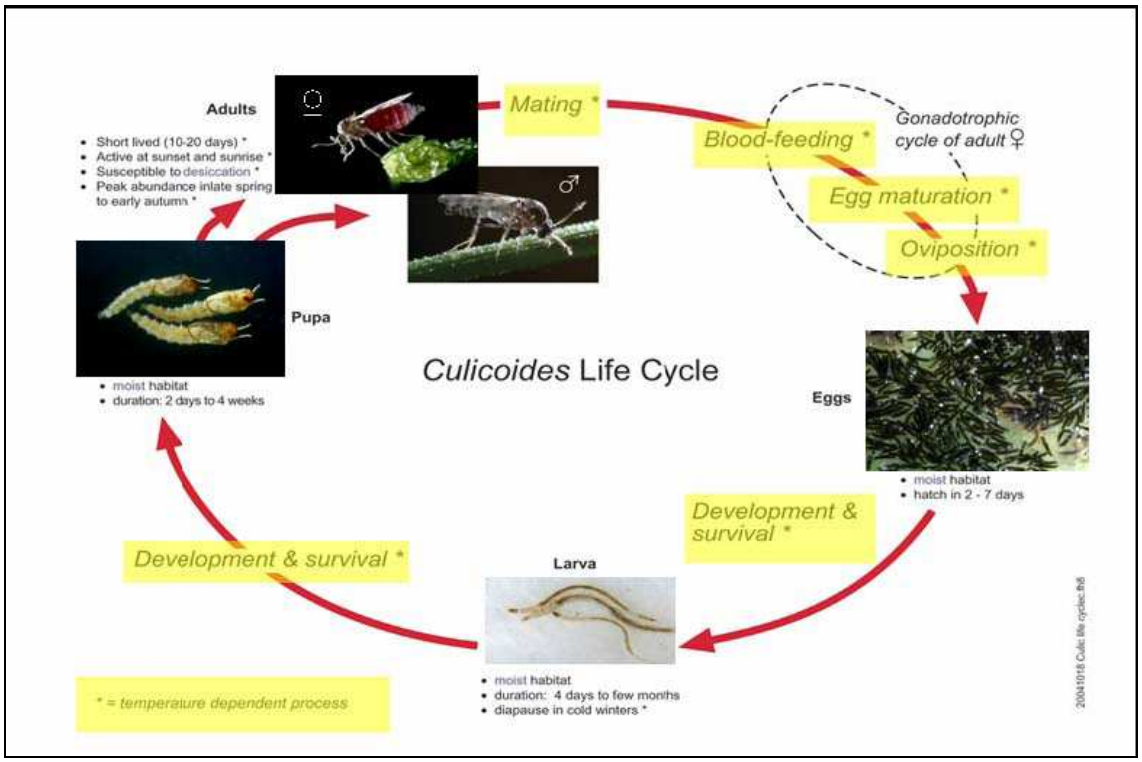
pulicaris ss seems less abundant than some of the other members of the complex such as *C. newsteadi* (Meiswinkel et al., 2008b).

These findings confirms the importance of Pulicaris and Obsoletus complexes midges even within latitudes where *C. imicola* occurs (Mellor, 2004) and demonstrates clearly that species endemic to the Palaearctic region are quite capable of transmitting BTV.

3.1.3.2 Life cycle

There are four main stages in *Culicoides* life cycle: egg, larva (4 stages), pupa and adults (Figure 1).

Figure 1: The *Culicoides* life cycle.



Source: Purse et al. (2005)

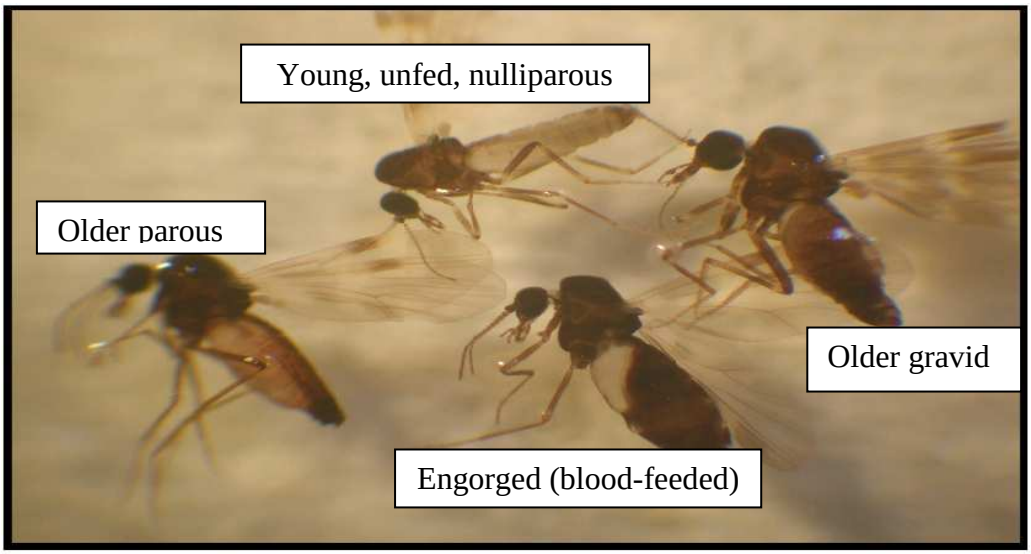
Generally, adult females are the ones that require blood meals at intervals of 3-4 days, to obtain protein to mature the ovaries and to lay between 30-450 eggs (Birley & Boormann, 1982). However, in some cases they may be autogenic i.e., able to develop eggs using nutrients retained from the larval phase or using alternative food sources such as nectars. Midges need to survive only 5 days to lay the first egg mass, but they must survive 10 days to transmit the virus. In general *Culicoides* eggs usually hatch in 4-5 days at 25 ºC. Low temperatures affect egg viability and time required for oviposition. In general, the time from egg until maturity is at least 3 weeks, depending on environmental temperatures (EFSA, 2007c).

All 4 larval instars are slim and smooth, characteristics that facilitate their movement through the semi-solid media they inhabit. Larvae of European *Culicoides* browse on bacterial films, microorganisms and nematodes. The vast majority of the larvae of *Culicoides* are pupated within 3.5 months. The lengthening of the larval cycle across latitudes appears related with temperatures and the number of daylight hours. The water content determines the suitability of a breeding habitat since they need moist to avoid desiccation. The pupa is a short-lived, non-feeding stage from which the adult midge hatches 2 days to 4 weeks later (EFSA, 2007c). All the members of the world-wide existing species of the genus *Culicoides* are rather small reaching often only a length of 1mm (Mehlhorn et al., 2007). Mating occurs mainly around sunset. Before *Culicoides* are able to mate, both sexes require several days to reach sexual maturity. Some *Culicoides* species are univoltine, having a single annual generation, but most appear to be multivoltine and 2-3 generations per year are common (McCarthy et al., 2008). From field data it has been estimated that the optimal temperatures for *C. imicola* development range between 18-38 ºC and for *C. obsoletus* between 11-27.5 °C (Dzhafarov, 1976).

3.1.3.3 The age status of *Culicoides* female midges

Dyce (1969) distinguished three stages of gonotrophic development in adult female *Culicoides* which are also a reliable indicator of their ages (Figure 2). The three stages include young, unfed, nulliparous females; older parous female and older gravid females. Parous midges have taken and digested a blood-meal and laid eggs, at least once previously, and so are the only specimens likely to harbour the virus (Mellor, 2004).

Figure 2: Gonotrophic stages of *Culicoides* females.



Adapted from Lucientes (2006)

Thus the age-grading of female *Culicoides* is an extremely useful tool in-field epidemiological study as it provides valuable information on the level of risk for BTV transmission. This topic will be explored in subsequent chapters.

3.1.3.4 Breeding habitat

It is of critical importance to recognise that each species occupies a specific niche in nature and that little overlap occurs amongst them. In fact, midge habitats are largely species-specific. More research is required to clarify the true breeding habitat for each species among the wide variety reviewed by Kettle & Elson (1980) and Meiswinkel, Venter & Nevill (2004). Of those, three main categories are presented.

1. Wet soils, between aquatic and terrestrial habitats - most world species of *Culicoides* breed in the muddy habitats, those that border water bodies; which range from fresh flowing streams to polluted stagnant pools, with varying degrees of acidity, alkalinity or salinity. The degree of light and the plant cover are additional factors determining the larval habitat. The soils will vary from the coarsest sand to the finest clay, with or without organic matter. Furthermore, *C. halophilus* occurs in coastal salt mud flats whereas, the closely related, *C. pulicaris* favours freshwater vegetated swamps. In Africa *C. imicola* favours moist (but not waterlogged) nutrient-rich clay soils.
2. Dung pats of large animals - in Europe, C. *dewulfi* breeds wherever cattle dung occur. The particularly close association between coprophilic *Culicoides* and livestock enhances their vector potential. All world species of dung inhabiting *Culicoides*, which includes almost half the world vectors of BTV, belong in the subgenus *Avaritia*.
3. Moist decaying vegetative material - the breeding sites for *C. Obsoletus* includes a wide variety of habitats, like rotten banana stumps (Mellor & Pitzolis, 1979), forest leaf litter, stagnant water and marshy areas (Dzhafarov, 1976), horse dung, and heaps of garden compost (Campbell & Pelham-Clinton, 1960). This plasticity in the ability of *C. obsoletus* to exploit a continuum of vegetative habitats explains why it occurs ubiquitously across Europe penetrating also into urban treed environment such as urban gardens (Conte, Goffredo, Ippoliti & Meiswinkel, 2007).

Accordingly, it is clear that half of the world vectors of BTV breed either in animal dung or in moist decaying vegetative materials and in some instances these breeding habitats are augmented by farming practices (EFSA, 2007b). Man might create breeding opportunities if the maintenance of livestock in the farmyard is neglected adding the daily water spill that creates muddy situations in which *Culicoides* will breed (EFSA, 2007c).

3.1.3.5 Heliophily/ umbrophily

C. pulicaris ss, like *C. imicola,* favours sparsely to moderately vegetated habitats that are open to sunlight (heliophily), but differ in that it occurs at more boreal altitudes and latitudes. For the Obsoletus complex they favour more densely vegetated habitats, experiencing reduced solar radiation (umbrophily). These characteristics will have a big influence in the length of the life cycles of *Culicoides* species (Conte et al., 2007).

3.1.3.6 Endophilly/ exophilly, exophagy/endophagy

It is often assumed that *Culicoides* are purely exophagic (i.e. feed outdoors) and that they remain outside (exophily) to rarely enter buildings (endophily). To date there is no clear information about resting sites for the main vector species (EFSA, 2008a). Nevertheless they spend well over 90% of their time resting (Mullens, Gerry, Lysyk & Schmidtmann, 2004).

In general, the biting activity is highest during crepuscular and nocturnal hours. However, during cooler periods of the year, they emerge from their resting places sooner in the day, when is still reasonably warm, to attack livestock while still at pasture. According with studies from Olbrich (1987), there is evidence of species (such as *C. obsoletus*) intensifying their attacks on overcast days when low-light conditions prevail. In this way it is possible that they follow cattle returning from their milking sheds and to accompany them indoors. Once inside these *Culicoides* would complete their blood feeding meal (Meiswinkel et al., 2008a).

The indoor activity, especially during winter time may extend the seasonal activity when outdoor activity is absent (EFSA, 2008a). Studies from Baldet and Delécolle (2006) verified that the indoor activity seemed dependent on temperature and not on intrinsic behaviour.

C. obsoletus, C. scoticus, C. chiopterus and *C. dewulfi* seem to be strongly endophagic. In Northern France, Baldet and his colleagues (2008) observed that in autumn adult *Culicoides* activity (mainly *C. obs*oletus and *C. scoticus*) was higher indoors than outdoors. These sample included a high percentage of parous females, with a relatively high proportion of them freshly blood feeded. Endophagic tendencies of the Obsoletus group seemed to be influenced primarily by external temperature and secondly by animal husbandry practices. The absence of an age category in *Culicoides* captured at the shed indicated that all *Culicoides* after engorgement exit the building to undergo oogenesis elsewhere.

Meiswinkel and his colleagues (2008c), in a study concerning the pronounced exophily of the Pulicaris complex, suggested that in areas where it predominates the housing of animals may prove beneficial. When conditions are warmer and calm, earlier in the season, outside catches can be high exceeding those inside. Therefore, stabling early in the season may protect animals from the *Culicoides* attack. In opposite, later in the season, when conditions are cold

and windy, outside catches are suppressed and inside catches increase. For that reason, stabling proves not to be beneficial.

3.1.3.7 Longevity

The longevity of adult *Culicoides* is dependent on the species and ranges from a few weeks or less, quite often, to 3.5 months in rarely cases. Studies in Germany showed that the active period of *C. obsoletus*, as a vector of BTV-8, was apparently longer and laboratory experiments proved that adult midges may live up to 3.5 months. These two peculiarities make it understandable that *C. obsoletus* may become the main vectors in Central Europe since it has a better chance to catch an agent of disease and also has more time to transmit replicated/ reproduced viruses during a higher number of bites compared with other vectors (Mehlhorn et al., 2007).

The proportion of older parous females within a population increases gradually as the season progresses to peak in late summer and autumn, precisely when outbreaks of *Culicoides*-borne pathogens also peak. Furthermore, a high parous rate is indicative of a high survival rate and increased longevity, which is essential for a pathogen to be successfully replicated within the insect vector and for it to be transmitted afterwards to a susceptible vertebrate host (EFSA, 2007c). Parous females are the older members within a population of *Culicoides* (meaning they have sucked blood and laid eggs at least once previously), and thus, are the ones most likely to harbour BTV in their salivary glands. Only BTV positive (BTV+) non-blood-feeded parous specimens indicate the replication of virus in the insect that may be transmitted subsequently to a ruminant host. Indeed only the presence of parous non feeding females will represent a high risk for BTV transmission (Baldet et al., 2008). Non engorged specimens that are BTV+ may simply reflect that they have sucked blood from an infected animal and not necessarily their capability of replicating BTV (Baldet & Delécolle, 2006).

3.1.3.8 Susceptibility to infection and transmission

The susceptibility of wild populations of *Culicoides* to infection by a blood-feeding meal is typically low (O'Connell, 2002). Within a vector species of *Culicoides*, susceptibility to infection and transmission of a particular serotype or strain is a genetically heritable trait. Consequently, results obtained by testing one or several populations of a suspect vector species of *Culicoides* shouldn't be extrapolated across most populations of that species (Mellor, 2004).

Probability of transmission from an infectious vector to a susceptible host is close to 100% (O'Connell, 2002). The efficiency in the transmission of BTV varies with vectors susceptibility, biting and survival rates, virus type and strain and environmental temperature.

The transmission from a vector to a host is more efficient when compared with the transmission from a viraemic host to the vector. Although the infection rates in vectors are low the high vector abundances and increased biting rates tend to compensate it.

In a study about the incursion of infected vectors to Switzerland, Racloz, Venter, Griot and Stärk (2008) explained that the number of new infections that would result from the introduction of a single infected vector specimen, into a completely susceptible population of hosts, relies on three main components:

1. Infection of the host that depends on vector density, vector biting rate, transmission rates from vector to cattle, cattle recovery rates and cattle death rates.
2. Longevity of the vector, which is dependent on the extrinsic incubation period (EIP), vector death rates, the time the virus is developing inside the vector amongst others.
3. Efficiency of transmission from host to vector which is the number of infectious bites per infected host. This is dependent on the transmission rates from cattle to vector and on the vectors death rates.

There is evidence of high rates of seroconversion to BTV in sentinel cattle in several years without high numbers of outbreaks, suggesting that many strains of BTV are non-virulent (Shimshony, Barzilai, Savir & Davidson, 1988). The transmission of replicating viruses is possible during a later blood meal after an initial infection, since it takes, at a temperature of 25 ºC, about 10-15 days to produce a sufficient number of transmittable viruses inside the female midge (Mehlhorin et al., 2007). Infected midge remains infective for life whatever is the titre, if the vectors competency and attack rate permit (EFSA, 2007b).

3.1.3.9 BTV and *Culicoides*

During a blood meal from an infected host, the virus passes into the lumen of the last part of the mid-gut. It then has to access the body of the insect before the potentially hostile environment in the gut lumen inactivates it or before it is excreted. Since this virus will be orally transmitted by the vector, it must reach the salivary glands with or without amplification in other susceptible tissues, multiply in them and finally be released with the saliva into the salivary ducts where it is available to infect a second vertebrate host during a subsequent bite. The details of this cycle are controlled by a range of interdependent variables (Mellor, 2004).

Due to the existence of various barriers to arbovirus infection in haematophagous insects, the proportion of *Culicoides* that is capable of virus transmission is lower than the proportion that can become infected (Paweska, Venter & Mellor, 2002).

3.1.3.10 Competence of *Culicoides*

After a “competent” adult *Culicoides* female feeds on a BTV infected ruminant host, many points can fail resulting in an “incompetent” midge. Vector competence refers to the ability of a vector to support virus infection and replication and/or dissemination (Mullens, Gerry, Lysyk & Schmidtmann, 2004). Vectorial capacity on the other hand refers to the ability of the vector population to transmit a pathogen. As result a competent vector may have a low vectorial capacity, due to low biting rates or survivorship, while a vector with low competence may be important (Mullens et al., 2004). Has a result, vectorial capacity provides a measure of disease risk, including vector competence, vector abundance, biting rates and incubation period (OIE, 2008b).

3.1.3.11 Prove vector status

According with Walton (2004) four criteria must be assessed to prove vector status. For BTV in particular are as follows:

1. The isolation of the BTV from wild-caught *Culicoides* specimens.
2. The demonstration of *Culicoides* ability to become infected by feeding upon a viraemic host.
3. The demonstration of *Culicoides* ability to transmit BTV by bite.
4. The confirmation, through field evidence, of the association of the infected midge with the vertebrate population in which infection is occurring.

3.2 History

The bluetongue disease was first described in 1876 in South Africa by an anonymous author and later referred by Hutcheon as “fever” and “malarial catarrhal fever of sheep”. Theiler, famous for his works on theileriosis, suggested in 1906 that this disease is the result of the action of a virus, similar to those described by Leoffler and Frosch in 1897 concerning the foot and mouth disease.

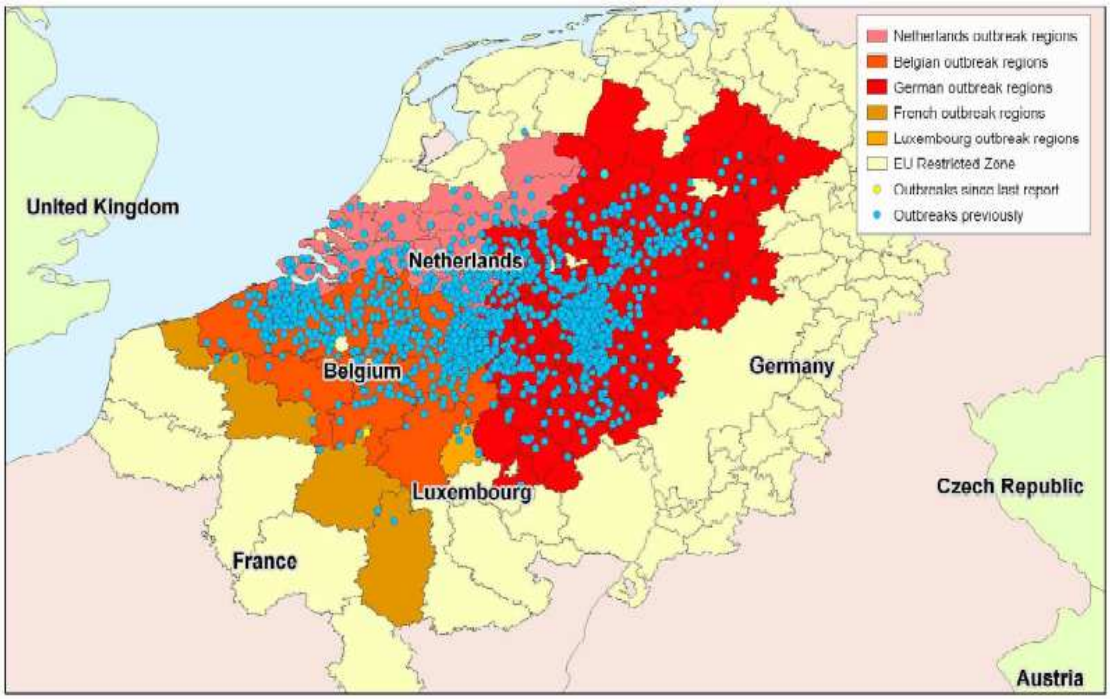
Originally, the disease was endemic in South Africa but was imported to other regions by transport of infected animals, especially to the Mediterranean basin (Mehlhorn et al., 2007).

3.2.1 2006 outbreak

Historically, BTD outbreaks in Europe were rare and short-lived. However, during the last 10 years, it has become firmly established in Southern Europe and, since 2006, has begun to occur in Northern Europe (Wilson & Mellor, 2008). In fact, in the latter half of 2006 an unprecedented outbreak of BTV-8 in sheep and cattle affected five member states (MS) in

Northern Europe. The first notification of BTV occurred in The Netherlands in the border area of Maastricht on August 14th and was confirmed by the Community Reference Laboratory at the Institute for Animal Health (IAH), Pirbright, on August 17th. Shortly thereafter, Belgium and Germany reported also outbreaks of BTV (on the 18th and on the 21st, respectively) and close to the same area (Baldet & Delécolle, 2006). Even with the reinforcement of the monitoring program, later, France and Luxemburg were also affected (Figure 3).

Figure 3: Bluetongue outbreaks in The Netherlands, Belgium, Germany and France.



Source: Rogers (2008)

This epizootic has probably caused greater economic damage than any previous single serotype of BTD. The total direct costs of the 2006 outbreak to the farming industry in the affected countries was estimated at € 1.1 million, while the direct costs of the 2007 exceeded € 150 million (Hoogendam, 2007).

BTD outbreaks can occur long distances outside the known range of the serotype responsible. The global BTV distribution is currently between latitudes of approximately 53º North and 34º south but is known to be expanding in the Northern hemisphere. *Culicoides* are responsible for the transmission of other important pathogens besides BTV and are doing so across an increasing geographic range (OIE, 2008b). Although rigorous import testing may help to mitigate the risk of BTD dissemination through livestock movements, *Culicoides* are capable of being transported by the wind for long distances and the movement of infected midges has been implicated as the most likely source of a number of outbreaks of bluetongue

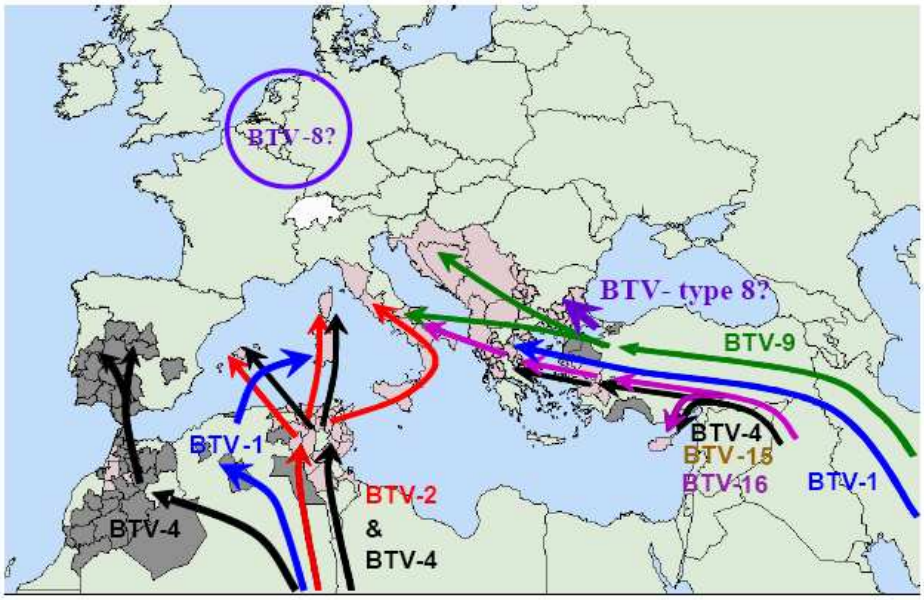
and other *Culicoides*-borne diseases (Sellers, Pedgeley & Tucker, 1977; Sellers, Pedgeley & Tucker 1978; Selers, Gibbs, Herniman, Pedgeley & Tucker, 1979).

The ability to define potential regions that are at risk for BTD epidemics is primarily based on the identification of the areas with competent vector species and on potential factors that could influence the temporal distribution of BTD outbreaks. The last includes the occurrence of viral incursion in surrounding countries, the introduction of new virus strains and the variation in presence of susceptible hosts (and levels of herd immunity). Still, there are many localities where populations of many of these known vector species can be found, yet there is no evidence of BTD (Tabachnick, 2004).

3.2.2 **Origin of the outbreak**

There are major gaps in the information related to the epidemiological situation of the different serotypes existing around EU. Genetic analysis of BTV isolated in Europe has shown that 6 serotypes of the virus (1, 2, 4, 8, 9 and 16) have entered the region since 1998. Possible pathways of introduction of BTV-8 (Figure 4) include imported infected ruminants, infected vectors introduced along with horses or with exotic plants and contaminated or unstable vaccines.

Figure 4: Map of the possible routes of introduction of the different BTV serotypes isolated in Europe since 1998.



Source: Mertens et al. (2004)

In general the experts consider first the role of infected ruminants in the introduction of new BTV serotypes and, additionally, the infected *Culicoides* transported by the wind in the subsequent spread in a free region (EFSA, 2007a).

Phylogenetic analysis have shown that the BTV-8 from the 2006 outbreak was most closely related to the 1982 chain of BTV-8 from West Africa (Meiswinkel, Goffredo, Leijs, & Conte, 2008d).

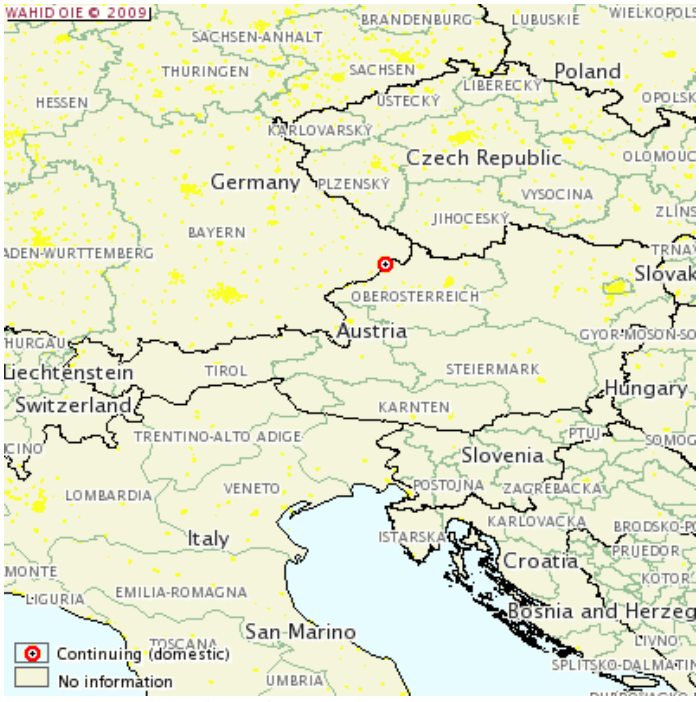
3.2.3 **Austrian situation**

The outbreak in wild birds in Austria of the *Flavivirus* named Usutu is one indicator, amongst others, of possible emerging threats in this country (World Animal Health Information Database [WHAID], 2008).

With the increasing awareness of the dynamics of BTD pathogen and the series of outbreak occurrences in neighbouring countries, in 2007, the first restricted zone was declared in the district of Bregenz (Vorarlberg) in Austria (European Commission [EC], 2007). In addition, the MHFY initiated a vector surveillance program, for the first time in the country, which was started in June 2007, and implemented by the Museum of Natural History in collaboration with the AGES (Silbermayr, 2009).

The first case of BTV in Austria was detected on the 31st of October 2008 in the district of Schärding (Upper Austria), during a routine screening on infectious bovine rhinotracheitis and infectious pustular vulvovaginitis (Figure 5).

Figure 5: BTV-8 outbreak in Schärding bordering Germany.



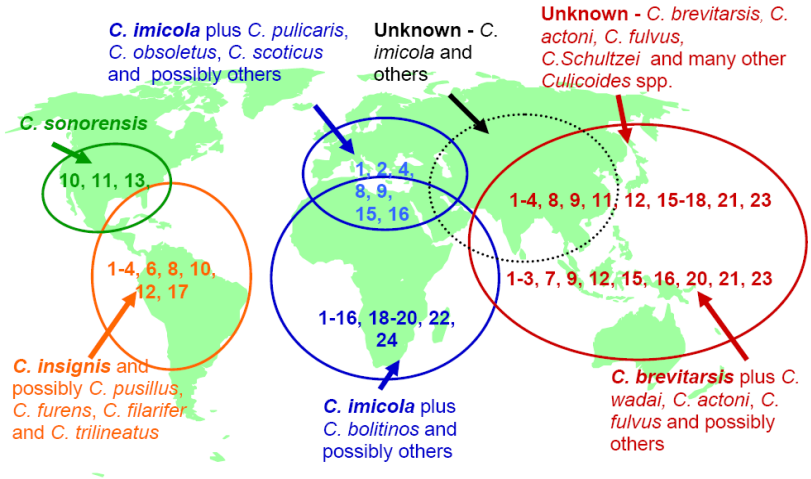
Source: WHAID (2008)

Later in the same year, on 11th December, another outbreak occurred in Bregenz. Both districts border Germany. In the end of 2008 the overall number of BTV-8 cases was 19 in Schärding and 2 in Bregenz, all in cattle. The disease was considered endemic from the 11th of May 2009 (WHAID, 2008).

These occurrences underline how pressing it is for Austria to establish a concise model to predict the occurrence of BTD vectors and identify flag areas, and risk zones, in which the disease can get established due to geographic, climatic and biological factors.

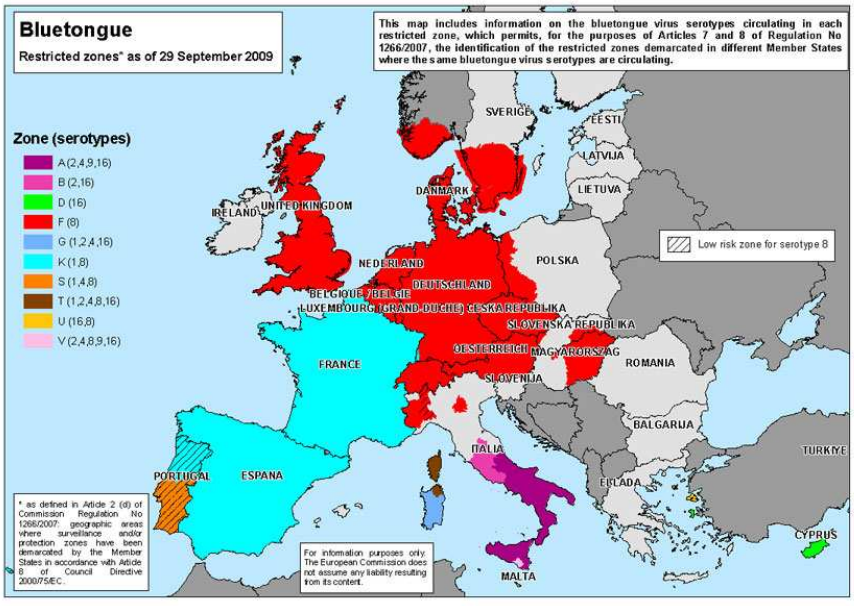
The distribution of each BTV serotype and vectors and the current European situation are illustrated in Figure 6 and 7 respectively.

Figure 6: Distribution of BTV serotypes and vector species worldwide.



Source: MacLachlan & Osburn (2006)

Figure 7: Restricted zones and the distribution of each serotype in Europe.



Source: EC (2009)

3.3 **BTD introduction, establishment and spread**

Epidemiology studies the distribution and determinants of diseases, including a spatial and/or temporal component (Carpenter, 2001).

There are 3 important and different steps in disease: introduction, establishment and spread. Introduction of a disease in a new area can occur through dispersal of infected midges, migration or through human intervention (e.g. commerce and travel). Establishment only occurs if the local conditions are permissive to agent permanence. Spread takes some time, as the agent needs to occupy all suitable niches. Its establishment point comes through the same processes that may have caused the original arrival, but always in habitats that are environmentally permissive. The natural flight of *Culicoides*, in the absence of any assistance of the wind, is on average of the order of up to 1 or 2 km per day (Purse & Rogers, 2008).

3.4 **Prevention and control**

According with the EU Directive 2000/75/EC (EC, 2000) when virus circulation is suspected or confirmed, MS must ensure the immediate notification of the competent authority and the official veterinarian shall, among other tasks, compile an inventory of places suitable for the vectors. In addition, the competent authority demarcates a protection zone and a surveillance zone, based on geographical, administrative, ecological and epidemiological factors connected with BTD and of the control arrangements. The protection zone has at least 100 km radius around the infected holding. The surveillance zone extends at least 50 km beyond the limits of the protection zone. The competent authorities from the MSs are responsible for the implementation of an epidemiosurveillance programme, including monitoring of sentinel groups and vector populations, in the protection zone.

There is no efficient treatment for BTD. Recovered animals pose no threat for transmission of the original infecting virus if they are confirmed to be virus-free (most readily by PCR prior to their movement) (EFSA, 2007c). Nevertheless in disease-free areas a quarantine and serological survey must be conducted. Due to the importance of the vector in BTD epidemiology, on both, disease free and infected areas, vector control is a priority. Whereas the air-borne and communicable diseases can sometimes be controlled by vaccination and quarantine, this is less clear for vector borne diseases (Takken et al., 2007).

Vaccination against BTV is a very important tool for the control of disease and is also used to achieve a safer trade in live ruminants in accordance with OIE standards and EU legislation. It is important to determine the waiting period relative to the use of attenuated and inactivated vaccines, before a vaccinated animal can be safely traded (EFSA, 2007b).

Protection risk management strategies when transporting animals through BTV infected countries or infected zones are of major importance in preventing BTD, particularly when avoiding the contact between vectors and hosts. These strategies include:

1. Treating animals with chemical repellents prior to and during transportation;
2. Loading, transporting and unloading animals at times of low vector activity (i.e. bright sunshine, low temperature);
3. Ensuring vehicles do not stop when movements occurs during dawn or dusk, or overnight, unless the animals are held behind insect proof netting;
4. Darkening the interior of the vehicle, for example by covering the roof and/or sides of vehicles with shadecloth;
5. Surveillance for vectors at common stopping and offloading points, to gain information on seasonal variations;
6. Using historical, ongoing and/or BTV modelling information to identify low risk ports and transport routes (OIE, 2008b).

Precise levels of protection provided by insecticide treatment and housing of stock is unclear and neither is likely to eliminate the risk of BTV transmission. However, insecticides treatments should be included in a set of measures designed to decrease biting midge densities. The efficacy of the insecticide depends on its activities, the protocol of application and the proportion of the herds in which it is applied. Even the most efficacious repellents are unlikely to significantly reduce *Culicoides* biting rates by more than a few hours.

If housing is not effectively sealed, housing livestock has little protective value, because *Culicoides* will enter buildings and bite cattle. It is advisable to control the vector densities by the elimination of most of the breeding sites instead of insecticide systematic use. It isn't prudent to treat the dung with insecticide; instead, regular cleaning of dung and housing, preventing sources of humidity are amongst other recommended measures (EFSA, 2007b).

3.4.1 **Surveillance**

The increasing occurrence and prevalence of vector-borne zoonoses are being attributed to environmental change and increased movement of livestock from one country to the other. In addition, vectors can rapidly spread across regions with favourable wind, with trucks and as ectoparasites on pets.

The open border policy of the EU requires the control of animal movements at the origin, with random checks at the destination (Takken et al., 2007). It is difficult to assess the risk of BTV transmission to susceptible animals moved through restricted zones given the difficulty of estimating parameters associated with vector biology (EFSA, 2008b).

Definition of zone boundaries and zone status, requires effective monitoring systems, to detect changes in the distribution of BTV, in response to changing climatic or environmental conditions. Zone boundaries are determined on the basis of monitoring results, geographical and environmental factors, and outputs of temporo-spatial models of vectors and virus distribution (Van Ark & Meiswinkel, 1992).

In the absence of clinical disease in a country or zone, its BTV status should be determined by an ongoing surveillance programme that may need to be adapted to targeted parts of the country at a higher risk, due to historical, geographical, climatic factors and *Culicoides* ecology. The purpose of surveillance is the detection of virus circulation in a country or zone and not the determination of the status of an individual animal or herd. Surveillance deals, not only with the occurrence of clinical signs caused by BTV but also with the evidence of infection in subclinical cases (OIE, 2008b).

There are several surveillance strategies, each aiming to a specific objective:

- The clinical surveillance aims at the detection of clinical signs of BT at the flock/ herd level.
- The objective of the serological surveillance is to detect evidence of BTV circulation. Samples should be examined for antibodies against BTV using tests prescribed in the Terrestrial Manual of the OIE.
- The isolation and genetic analysis of BTV from a proportion of infected animals constitutes the virulogical surveillance, providing information on serotype and genetic characteristics of the viruses concerned.
- The sentinel animal programme primary purpose is to detect BTV infections occurring at a particular place. For instance, sentinel groups may be located on the usual boundaries of infected zones to detect changes in distribution of BTV. In addition, sentinel animal programmes allow the timing and dynamics of infections to be observed.
- Finally, and the most important for this work, the vector surveillance allows the identification of potential vector species accurately, although many such species are closely related and difficult to differentiate with certainty. The main purpose of vector surveillance is to define high, medium and low- risk areas and local details of seasonality; by determining the various species present in an area, their respective seasonal occurrence, and abundance. Vector surveillance has particular relevance to potential areas of spread. Long term surveillance can also be used to assess vector suppression measures. The most effective way of gathering this information must take in to consideration the biology and behavioural characteristics of the local vector

species of *Culicoides*. The operation of vector surveillance sites at the same locations as sentinel animals is advisable (OIE, 2008b).

According with the OIE (2008b), surveillance of BTD should be in the form of a continuing programme. The entomological surveillance systems allow the achievement of field data for the continuous refinement of the distribution map of *Culicoides* together with the detection and elucidation of the prevalence of new suspected vectors. It also elucidates the seasonal dynamics of all vectors in order to identify zones that are seasonally vector free. And at last, but not the least, it aims to collect samples for the detection of BTV in *Culicoides* during outbreaks of the disease. These goals can only be achieved with data that are both comparable and sensitive. In any BTD surveillance system, the principal aim is to capture adult *Culicoides* in the near vicinity of vertebrate hosts, with powerful traps (to enhance surveillance sensitivity at low *Culicoides* levels and, furthermore, to increase the number of midges captured for virus isolation studies) (Goffredo & Meiswinkel, 2004).

According with the entomological programme, a country or zone is said to be BTV free for instance if there is no evidence of *Culicoides* likely to be competent BTV vector in its geographical area. BTV seasonally free zone is a part of an infected country or zone for which, for a part of the year, surveillance demonstrates no evidence either of BTV transmission or when less than 5 parous *Culicoides* are found in a light trap suspended outdoors for one night (Meiswinkel et al., 2008a). This period ends immediately if current climatic data, or data from the surveillance programme, indicates an earlier resurgence of activity of adult *Culicoides* likely to be competent, amongst other reasons (OIE, 2008b). This criteria could be augmented by subjecting also the parous midges to Polimearse Chain Reaction (PCR) assay, as the specimens from the previous season are more likely to be affected than the new ones (EFSA, 2007b).
MS shall provide scientific data that explain the epidemiology of BTD in the region concerned and adapt the surveillance strategies (OIE, 2008b).

3.4.1.1 Capture of *Culicoides*

According with Goffredo and Meiswinkel (2004), *Culicoides* can be captured using several methods. The adults are most easily captured using various light trap models (these vary in power, colours and additional attractants), truck traps, aspirators, bait traps and also emergence traps for the sampling of larval habitats. In addition, the immature stages can be harvested from various moist habitats. Each method has specific advantages and disadvantages.

The number of *Culicoides* spp. caught per night in a light trap is proportional to the size of the local population, the activity rate and by the efficiency of the trap (Baylis, O'Connell & Purse, 2004).

Culicoides activity is also dependent on light levels. That is why trap efficiency is reduced by other light sources such as moonlight. The use of a suction device in light traps reduces its efficiency with an increase in wind speed. Trap catches outside a stable are reduced at higher wind speeds, while those, inside the stable, are not (Baylis et al., 2004).

The presence of males in collections is considered to indicate the presence of breeding sites nearby (EFSA, 2008b). In a study from Mehlhorn and his colleagues (2007), the relation of the caught males and females was always favourable to females from double up to 40 times more females. This data indicate that mating apparently does not occur close to the feeding places. Also the number of fed *Culicoides* was higher than that of unfed.

3.4.1.2 Identification of *Culicoides*

The morphology-based taxonomic classification of about 6000 species of the dipteral family *Ceratopogonidae* is difficult. Many authors refer that the species of the genus *Culicoides* can be classified against species of other genera of *Ceratopogonidae* by a characteristic of light and dark spots on the wings (Balczun, Vorsprach, Meiser & Schaub, 2009). Within this genus, the species are classified into the subgenera by a different criteria, e.g. in males by characteristics of the genitalia and in females by the distribution of the spermatheca. However, according to molecular biological investigations, some subgenera contain three or more species complexes (Meiswinkel, Gomulski, Delécolle, Goffredo & Gasperi, 2004a). For example, *C. obsoletus*, *C. scoticus* and *C. montanus* can only be differentiated in the female form by molecular markers (EFSA, 2007b).

3.5 Risk factors

The central role of the insect in BTD epidemiology makes the prevalence of the disease dependent on ecological, climatic and environmental characteristics. These factors exert a substantial influence on the transmission potential of BTD. The examination of climate and vector population patterns reveals potentially powerful relations for all vector-borne diseases. These associations can be used to make hot spots on a spatial scale using real-time climate data (Silbermayr, 2009).

The geographical distribution and vectorial capacity of *Culicoides* vector populations determine the spatial and temporal incidence of BTV transmission. These populations are in turn influenced by climatic factors such as temperature, rainfall, wind and moisture (Mellor et al., 2000). The presence and density of ruminant's hosts is also of great relevance. In fact, in

2008 in a study from Takken and his partners, *C. obsoletus* was found in large numbers at the livestock farms than at the other habitats and was found more often at the farms with higher density of farm animals.

3.5.1 **Temperature**

Many factors affect vectorial capacity, but none is more potent or predictable then temperature, since BTD vectors are poikilotermic.

The air temperature (TAIR) is usually used to study vectors dispersion since it is an estimate of the air temperature a few meters above the land surface. Whereas the Land surface Temperature (LST) is a general index of the apparent environmental surface temperature (Purse et al., 2004b).

Temperature is the main climatic factor to modulate BTV transmission since it has a complex effect on many parameters that determine *Culicoides* vectorial capacity such as on the gonotrophic cycle (Linley, 1966), survival from the immature stages to adulthood (Bishop, McKezie, Barchia, & Harris, 1996), vector breeding, adult survival (Hunt, Tabachnick & McKinnon, 1989), biting rate (Mullens & Holbrook, 1991), duration of the EIP and vector competence (Wittmann, 2000).

Temperature also governs adult feeding frequency and the opportunity to acquire or transmit BTV because during climatically favourable periods, *Culicoides* females feed every 3-4 days. For *C. obsoletus* a 4.0-4.9 day's interval between blood meals was calculated but may be reduced further at climatically optimal periods. Increased blood feeding frequencies, coupled to high vector densities, promote the more rapid dissemination of the virus amongst other susceptible vertebrate hosts (EFSA, 2007b). In the adult midge body, the temperature also influences virus replication (Mullens et al., 2004).

Temperature is a driving force for immature vector developmental rates. This influences the number of generations produced and the adult population size (Mullens et al., 2004). For instance, to halt larval development or to induce diapauses both low day and night temperatures, and a decrease in the length of the photoperiod, are required (Baldet et al., 2008). Larval mosquitoes, stressed by nutrition or high temperature, emerge as smaller adults.

Understanding the mechanism by which a virus overwinters is of crucial importance in defining the basic epidemiology of the virus (White, Wilson, Blair & Beaty, 2005). Virogenesis is faster at higher temperature, thus the corresponding EIP varies dramatically with it. For example constant temperatures, of about 27-32 ºC, may allow virus titres to peak in 4 to 8 days; while at 21 ºC the midges might require 16 to 22 days. Nevertheless, highly temperature decreases daily survivorship and exerts a negative effect (Mullens et al., 2004). The optimal range for BTV transmission lies between 27-30 ºC, even though *Culicoides*

maybe active at temperature close to 10 ºC and viral replication starts at 15 ºC (EFSA, 2007b).

Vector profit from higher summer and winter temperatures by extending their reproductive season and greater ability to disperse (Meiswinkel et al., 2008a). In experiments from Goffredo, Romeo, Monaco, Di Gennaro & Savini (2004) *C. obsoletus ss* and *C. scoticus*, survived for up to 92 days at 17-25 ºC without a blood meal. The same species also recovered with ease after being kept for 10 days at 4 ºC. This lengthy life-span and resistance to low temperature plays an important role in BTV resistance.

C. obsoletus probably adapted to cold, requiring relatively low temperatures for optimal development and survival (Purse et al., 2004b). Accordingly, Obsoletus complex tolerates temperatures as low as -29 ºC. This implies that diapausing larvae of the Obsoletus complex maybe equally tolerant enabling them to overwinter in more northerly areas that are climatically harsh (Conte et al., 2007). Overwintering allows the maintenance of the virus between seasons with the resulting endemicity of the disease. Many mechanisms have been reported has possible for the overwintering of BTV but many gaps remain in this subject.

3.5.2 **Precipitation**

Rainfall may transform a vector population that is simply maintaining BTV, to one explosive transmission within a few days. This is achieved mainly through increased survival of adult insects. Generation rates increase with the availability of breeding sites, and increased adult survival rate is related to avoidance of desiccation; all intimately related with rainfall (Murray, 1986). It is the survival of adult vectors that determines vector dynamics and therefore transmission of BTV (Ward & Carpenter, 1995b).

Lack of rain and the concomitant low soil moisture may desiccate larvae. However, at annual average rainfall levels above 700 millimetres (mm), soils become leached of nutrients and so, unsuitable for breeding sites (Meiswinkel, Labuschagne, Baylis & Mellor, 2004b). Low relative humidity (RH), at high temperatures in the other hand, causes low saturation deficit, which will desiccate adults (Baylis et al., 2004).

3.5.3 **Wind**

Midges exhibit active movement, irrespective of wind direction, when wind speed is below 3 meters by second (m/s) and stay sheltered at wind speeds above 11 m/s. Not only wind intensity, but also, wind direction is important factors for dispersal (Hendrickx, Ducheyne, De Groot, Codina & Gilbert, 2008).

Across the sea, *Culicoides* spread has been suggested over distances up to 700 km (Braverman & Chechik, 1996). This is due to the relative smoothness of the sea surface

offering no barrier to horizontal air flows. Over land, due to the roughness of terrain, wind spread occurs mainly in an interrupted pattern where large distances may be covered between the start and the end of the epidemic at a rate of 15 km per day, mostly within a range of 31 km (Hendrickx et al., 2008).

When characterizing the movement of insect vectors Sellers (1992) observed two types of flight, a short distance flight (10 m to 5 km) that occurs in any direction and at low or zero wind speed, and a long distance dispersal, up to several 100 km, that occurs at wind speed greater than the unaided flights speed of *Culicoides* and where midges are carried by the wind. These movements may be terminated by many factors either actively, or because the wind drops and may therefore introduce BTV into regions remote from the source. It appears that after the introduction of the virus in the initial herd, a sufficiently high number of herds can become infected through local active spread. It results in a sufficiently high number of infected midges in the population, yielding a sufficiently high number of infective wind events, enabling a long range spread of these midges (Hendrickx et al., 2008).

These stress the importance of efficient monitoring, especially at the start of the epidemic, preventing the dispersion of huge amount of vectors by the wind and the long range spread of the epidemic. The drop down in large numbers create a local cluster which then further grows through local spread as explained in previous chapters.

3.5.4 **Aridity**

Aridity index is based on the ratio of precipitatiion to potential evapotranspiration. Low values indicate increasing dryness, whereas higher values indicate increasing wetness (Lincoln, Boxshall & Clark, 2003). According with studies from Conte and his coleagues (2007) in Italy, the majority of the large collections of the Obsoletus complex came from municipalities with a higher aridity index.

3.5.5 **Slope**

In an undulate topography, where the slope exceeds 5º, water overflow will induce rapid desiccation of the soils surface layers (Conte et al., 2007). *C. imicola* appears where topography is flat or only moderately undulate. Conversely, *C. obsoletus* occurs more ubiquitously, penetrating into terrain that is either flat or strongly undulate and up to elevations of 2000 m (EFSA, 2007c).

3.5.6 **Altitude and latitude**

Digital elevation (DE) is an obvious correlate of many other important variables, such as temperature, rainfall, land-cover and wind exposure (Purse & Rogers, 2008). Latitudinal effects serve to increasingly shorten the vector season with fewer vector generations per

season. Its impact on vectors ecology is important to avoid overwintering and, hence, suppressing the endemisation of BTV (EFSA, 2007b).

3.5.7 **Moisture, Normalized Difference Vegetation Index (NDVI) and Middle Infrared reflectance (MIR)**

Moist soil enriched with organic matter appears to be the most suitable habitat for larval development (Braverman, Galun & Ziv, 1974). Optimal range of relative humidity ranges from 65-80% (at 15-27 ºC) **(**Reynolds, Chapman & Harrington, 2006). *Culicoides* cannot survive when temperature is consistently lower than 7 ºC in combination with a relative humidity lower than 30% (Wittmann, 2002).

NDVI is widely used within the field of remote sensing, and is specifically a measure of chlorophyll abundance, but it is also correlated with soil moisture, rainfall and vegetation biomass, coverage and productivity (Campbell, 1996). According with Braverman and his colleagues (2004), outbreaks are more likely to occur when moist conditions prevail (indicated by high NDVI) in a particular month or up to four months previously.

C. pulicaris prefers microclimates with high stable levels of moisture, for optimal survival and development. A study from Blanton and Wirth (1979) revealed that NDVI variables were the most important determinants of the distribution of *C. pulicaris*. This is consistent with its breeding site requirements which are listed as wet soil and bogs.

MIR is correlated with the water content and surface temperature. Since this spectral region contains both reflected (short wavelength) and emitted (longer wavelength) radiation, it is also associated with tree canopy density and structure (Purse & Rogers, 2008). According with studies from Purse and his partners (2004b) in Italy, for *C. newsteadi*, high values of MIR were associated with minimum temperatures, probably reflecting high thermal requirements for development of this species.

Climate and weather models can provide a measure to predict vector distributions and epidemic risk. It is essential to understand the details of the influence of many other factors on the vector capacity of a species in order to provide accurate prediction and mitigation of potential epidemic risk (Tabachnick, 2004).

3.6 **Modelling**

The purposes of the modelling is to improve the understanding of the biotic and abiotic determinants of the distribution of the vectors, identify the limits of the distribution, define new areas at risk and areas that might remain disease free and finally to identify the way that the distribution might change under different scenarios (Baylis et al., 2004).

Mathematical models are useful for the study of complex phenomena, like the population dynamics of infectious agents, because models show how separate measurements can be seen as manifestation of the same underlying processes (De Jong, 1995). They also assess the impact of various measures to be taken for minimizing the risk of introduction of BTV (EFSA, 2007a). The BTV risk model demonstrates that under favourable climatic conditions, BTV can spread aided by the presence of suitable vectors. These vectors are indigenous and not exotic. It is the interplay of statistical and biological insights that makes the fusion of the two approaches so productive (Purse & Rogers, 2008).

Modelling efforts and improved environmental descriptive tools, including GIS technologies, have helped in demonstrating that environmental factors should be examined more closely (Mullens et al., 2004).

3.7 **GIS**

Many infectious diseases have an obvious seasonal determinant, so satellite data should capture important elements of the seasonal cycles (Purse & Rogers, 2008). These GIS methods provide robust opportunities for processing, analyzing and displaying spatially based environmental data concerning vector borne diseases (Kitron, 1998). GIS analysis also facilitate the interpretation of spatially rellated environmental factors that influence vector population occurrence and abundance (Baylis, Mellor, Wittmann & Rogers, 2001).

3.8 **Risk maps**

A risk map is a representation of how an area (a region, state or continent) would look if sampled at all points for disease or a vector under study. It is not possible to prove whether or not any predictive risk map is 100% accurate since there is never enough data. However, over the course of time, sufficient observations are accumulated to give confidence in the capabilities of the risk maps to identify areas at risk of a disease. The other requirement is a prediction of the time of greatest risk, or the onset of risk seasonality.

The key variables contributing to spatial risk maps are examined to see if they explain some biological processes. Since these variables have an element of seasonality, it might be possible to make some predictions about the increasing or decreasing risk in an area through time (Purse & Rogers, 2008).

4 MATERIAL AND METHODS

4.1 Data and software

Risk map construction requires data as well as specific statistical and mapping software. The building process can be summarized in four main parts, starting with the identification of data sources and collection, data processing, followed by the statistical modelling and finally the construction of the risk maps.

For data processing and graphics the software applied consisted of Microsoft Office Excel and Access© 2007; the statistical computing, graphics and risk mapping were executed with R 2.8.1 and ArcGis 9.2, in a Windows XP© environment.

4.2 Data sources and collection

4.2.1 Data sources

The analysis of BTV vectors occurrence takes several aspects in to consideration, besides the amount of *Culicoides* trapped and their identification. Information concerning environmental and topographical conditions has also major importance in this process. Six main data sources were needed for this study:

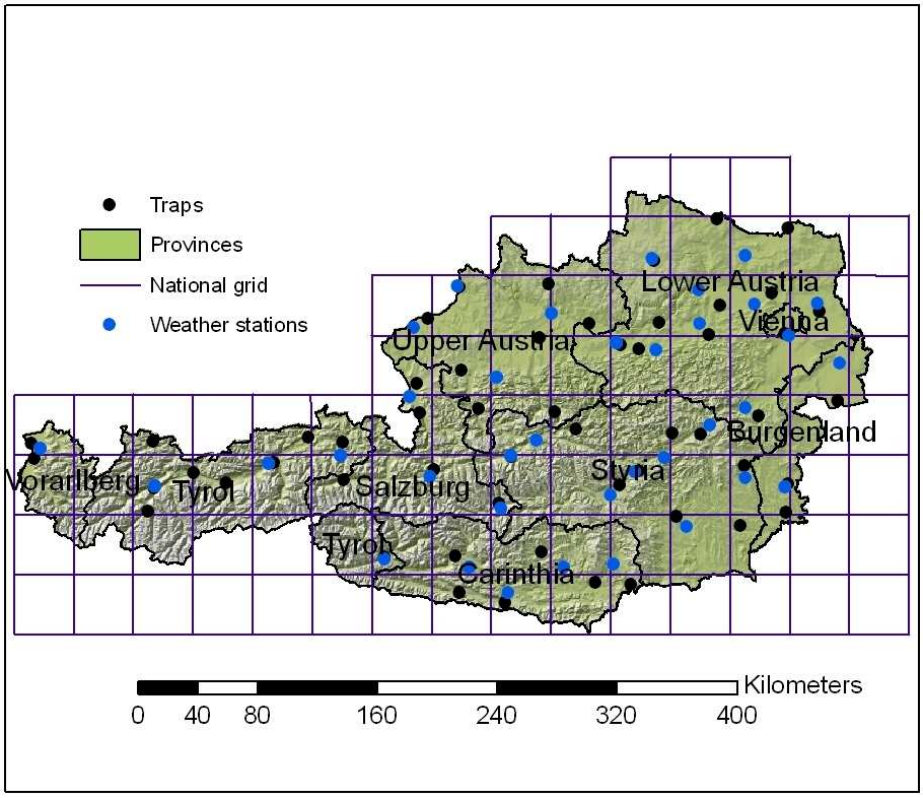
1. the Austrian surveillance system that provided the entomological information;
2. the Verbraucherinformationssystem (VIS-data) from Statistik Austria from which the animal density was created;
3. the Austrian Central Institute for Meteorology and Geodynamics (ZAMG) for the weather stations data;
4. the Umweltbundesamt Federal Environment Agency that provided the land cover information;
5. the European Distributed Institute of Taxonomy for the (NDVI);
6. the Consultative Group for International Agriculture Research - Consortion for Spatial Information (CGIAR-CSI) where the Digitalized Elevation Model (DEM) was obtained.

4.2.2 Data collection

In 2007, the project on vector surveillance system in Austria was started. According to Sehnal and his colleagues (2008), the whole country was divided into grid cells of 40 km x 40 km and approximately, one farm per grid cell was selected randomly as trap location (Figure 8). The sampling criteria consisted of having more than ten individuals of cattle, sheep or goats, within a moderate altitude (that ranged from 116 to 1192 meters), and the willingness of the farmer to cooperate. Cells including the borders of the country with only partly areas of

Austrian territory and other areas covering alpine regions were excluded from the study since there was no information available for the entire cell and some of the places were not so suitable for the vectors. Fifty four Onderstepoort-type blacklight-traps were mounted as close as possible to the animals, but out of their reach, in a weather-proof area outside the stable and were activated every day from Monday to Sunday from sunset to sunrise. The collection beaker contained a liquid composed of water and few drops of soap sud in order to break the surface tension. The water was replaced with 75% ethanol in colder nights, with minus degrees. Every Monday, the weekly sampled material was transferred into a plastic transport beaker containing 75% ethanol and forwarded to the Diptera collection at the Natural History Museum in Vienna. Then they were treated according to the recommendations of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, the OIE centre of reference for BTD. Samples were divided by catch and according to their amounts. Samples with less than 500 specimens were treated entirely or divided in subsamples if bigger then 500. The midges were assigned to species complex (Obsoletus and Pulicaris complex) with the determination keys particularly from Delécolle (1985).

Figure 8: Map representing the Austrian surveillance system with the national grid, the locations of the traps and the weather forecast stations in each Province.



The vectors in study were mainly the Obsoletus and Pulicaris complexes, taking in to consideration their prevalence among the other vectors from the genera *Culicoides* (*Culicoides* spp.) and also the *Culicoides* spp. itself to achieve an overall idea of the BTV vectors in the country. As was mentioned above, the species level identification was not possible. So whenever it's referred Pulicaris or Obsoletus, it's always referred to their complexes and not the species themselves.

4.3 **Data processing**

4.3.1.1 Entomological surveillance data

Given the fact that the main goal of this work is to produce the risk maps that help to predict the BTV vectors occurrence, the weekly results from the entomological surveillance needed to be gathered on a monthly basis. Since the number of vectors in one month might be related to the events in the month(s) before, the study in trimesters/seasons was also carried out to obtain more information. The time period considered ranged from April to September 2008 because this was the period with data available at AGES and allocated to this study. Thus, the average of the catches for each trap in each month were calculated and added to 6 monthly data sets. With the monthly datasets the season datasets were created with the information from April to June belonging to the spring and from July to September referring to the summer. By this time there were 5 datasets with 54 records each, and 1, for June, with only 53 (because one of the traps had no record) for the monthly analysis and two more datasets for the spring and summer, each with 54 records. These datasets contained the code of the trap, the month or the season, the averages of the amount of BTV vectors trapped and the coordinates of the georeference of the traps.

4.3.1.2 Weather stations data

The corresponding weather data, derived from forty five neighbouring weather stations in the ground, was provided in Excel sheets, each containing the information for one meteorological station on a daily basis. The exact locations of all meteorological stations were georeferenced so that the nearest from the trap was always chosen. The monthly and season information was calculated so that the following weather variables were obtained:

- the averages of the minimum, maximum and mean temperature (ºC);
- the absolute minimal and maximal temperatures (ºC);
- the average, minimum and maximum relative humidity (%);
- the average and maximum wind speed (m/s);
- the total precipitation (sum of daily precipitation in mm) and the days with rain.

Each trapping month and season was attached with the corresponding weather measurements.

4.3.1.3 Animal density

Animal density was assumed constant for all the study period and refers to all the domestic ruminants registered, such as cattle, sheep and goats, in January 2009. Since this information was not available for all the country, for some trap locations, data was obtained by using the corresponding kernel density with 5 km search radius and 1 km cell size and the value for each trap was extracted to the dataset by AGES.

4.3.1.4 Altitude

A digital elevation model in the Lambert Conformal Conic coordinate system with a 100 m resolution was used and the altitude for each trap location was extracted to the dataset by AGES.

4.3.1.5 NDVI

For the NDVI information six grids with 0.1x0.1 degrees resolution in WGS84 obtained from satellite images from National Oceanic and Atmospheric Administration - Advanced Very High Resolution Radiometer (NOAA-AVHRR) over the entire globe were provided by AGES, and the value for each trap extracted to the corresponding dataset.

4.3.1.6 Land cover

Land cover classification, from remotely sensed images, were provided by AGES in a main table and later were reduced into three main classes (urban, agriculture and forest zones) to simplify the analysis.

After the preparation of the datasets they were computed with R. The main structure of the datasets can be seen in Table 1.

Table 1: Variables in the datasets.

Variables in a month or season	Description
ID	Identification code of the record
Month/ Season	From April to September/Spring or Summer
Trap code	Code of the trap
Station code	Code of the weather forecast station

Table 1: Variables in the datasets (continuation).

Coordinates		To georeference the trap
Average of the amount of vectors trapped	*Culicoides* spp.	Average of the catches by trap
	Obsoletus complex	Average of the catches by trap
	Pulicaris complex	Average of the catches by trap
Temperatures in Celsius degrees (ºC)	Avg_tmin	Average of the minimum temperature in
	Avg_tmean	Average of the mean temperature
	Avg_tmax	Average of the maximum temperature
	Min_tmin	Absolute minimum temperature(which is. the lowest value of the minimum temperature)
	Max_tmax	Absolute maximum temperature (e.g. which is the biggest value of the maximum temperature)
Relative humidity in percentage (%)	RH_min	Minimum of the relative humidity
	RH_avg	Average of the relative humidity
	RH_max	Maximum of the relative humidity
Precipitation in millimetres (mm)	Precipitation	Total precipitation in ml
	Days_rain	Days with rain
Wind in meters by second (m/s)	Wind_avg	Average of the wind speed
	Wind_max	Absolute maximum wind speed
Animal density		Cattle, sheep and goats.
Altitude (m)		Altitude of the trap
Land cover		Three classes: Urban (“U”), agriculture (“A”) and forest (“F”) zones
NDVI		Average of NDVI.

4.4 **Statistical modelling**

The process can be summarized in three stages: (1) the model reduction and the estimation of the parameters, (2) the model validation and finally (3) the careful confirmation of the model assumptions.

All the 16 variables except for the land cover (factor) were analysed as numeric (Table 2).

Table 2: Variables in the beginning of the model reduction and their type in the statistical analysis program.

Variables	type
Avg_tmin	Numeric
Avg_tmean	Numeric
Avg_tmax	Numeric
Min_tmin	Numeric
Max_tmax	Numeric
RH_min	Numeric
RH_avg	Numeric
RH_max	Numeric
Precipitation	Numeric
Days_rain	Numeric
Wind_avg	Numeric
Wind_max	Numeric
Animal density	Numeric
Altitude	Numeric
Land cover	Factor
NDVI	Numeric

Since the goal was to model the relation between vectors occurrence (the dependent variable) with several variables (the independent variables), the multiple linear regression model was selected. This was achieved by fitting a linear equation to the observed data. Understanding the epidemiology of the disease was essential to preselect candidate predictors. Formally, the model for multiple linear regression, given n observations is:

Equation 1: Formula of the multiple linear regression model.

$$Yi = \beta 0 + \beta 1 xi1 + \beta 2 xi2 + \varepsilon i \qquad \text{for } i = 1, 2, \ldots n,$$

Yi is the response variable; xi1,…,xik are the explanatory variables; β0,β1,…,βk are the regression parameters and εi, i=1,…,n, are the random errors.

The fitted values estimated the parameters of the population regression line by the method of least-squares (Ugarte, Militino & Arnholt, 2008).

The significance level p selected was 0.05 taking in to consideration the fact that the scientific community, as a whole, has decided that the vast majority of these tests should be carried out at this level. Therefore, if the model has predictive capability, $p < 0.05$, the difference is said to be statistically significant (Hoffman, 1993).

The modelling process started with the full equation, which is with all the variables in study, and successively dropped one variable with the biggest non significant p value at a time. This type of model reduction is called backward procedure and it attempts to identify a small subset of factors that relate significantly to the outcome. So, for each step of the model reduction, all the variables were compared on how they help explaining the variability of the occurrence of BTV vectors. After each drop the process is repeated for those variables that still remained in the model. If all test results are significant, the full set of relevant variables is retained in the equation (Katz, 1999).

Once the multiple regression fits the data, the estimates were obtained for the various parameters of interest, and accessed in terms of their contributions to the prediction in the vectors occurrence (Hoffman, 1993).

After achieving the estimates for the parameter, in a second step, the goodness-of-fit was accessed by two main elements: adjusted R Square and Bayesian Information Criteria (BIC), this step is called model validation. Adjusted R square measures the proportion of the variation in the dependent variable, the occurrence of BTV vectors, accounted for by the explanatory variables. The bigger the adjusted R Square, the better the model. In the same process, several competing models were ranked according to their BIC, with the one having the lowest BIC being the best (Ugarte et al., 2008).

Before finishing the statistical modelling process, the analysis of the model assumptions was checked by the residuals examination. Several transformations were made to improve and bring the data closer to fit the model. Among all the transformation tried in the response variable, such as log or square root, the log+1 transformation was the best one. The models were refit and the residuals assessed in a manner to ensure that the model assumptions were approximately all met. Therefore the assumptions of linearity, independence, constant variance, normality and absence of outliers were all carefully examined.

Independence between observations was evaluated based on the information of the design and data collection procedure. The assumptions of linearity and homocedasticity were evaluated based on plots with the fitted values against the residual. Probability plots, also known as quantile plots (QQ), were used to check for normality after a log transformation. To check for outliers the method of Cook's distance was selected.

4.5 **Construction of the risk maps**

The models were applied in ArcGIS.

The grids for NDVI, the land cover, DEM and the animal density were supplied by AGES. Since there were only sample points for the average of the amount of vectors trapped and the significant meteorological variables, ordinary Kriging interpolation of the Geostatistical

Analyst module of Environmental Systems Research Institute (ESRI) - ArcGIS was used to estimate the values for each grid cell and create a continuous surface.

The equations of the models achieved in the previous step (statistical modeling) were introduced in the raster calculator a Spatial Analyst tool from ArcGIS, to achieve the grids for the whole country with the estimated number of complex species for each month and season.

5 RESULTS

5.1 Descriptive statistics of the data from the Surveillance System

From April to September 2008, a total of 1257 samples were taken from 54 traps distributed along the 9 Austrian Provinces (Table 3). The average number of samples by trap is lower in Burgenland (20) and bigger in Vienna (26), the remaining provinces have between 23 and 24.

Table 3: Number of traps and samples by Province.

Provinces	Nº of traps	Nº of samples	Avg Nº of samples by trap
Vienna	1	26	26
Vorarlberg	2	47	24
Burgenland	3	60	20
Salzburg	5	118	24
Carinthia	6	144	24
Upper Austria	8	187	23
Styria	9	214	24
Tyrol	9	206	23
Lower Austria	11	255	23
Total	54	1257	23

During the study period, 4 539 297 specimens of the genera *Culicoides* were analysed. Within this genus, 91.7% were assigned to the Obsoletus complex, 4.6% to the Pulicaris complex. The remaining 3.7% were assigned to species not belonging to any of these complexes or were not able to be determined to complex or species level (Table 4).

Table 4: Number of BTV vectors trapped from April to September 2008 and the correspondent percentages.

Species/Genus	Number of midges trapped (%)
***Culicoides* spp.**	4539297 (100)
Obsoletus complex	4162499 (91.7)
Pulicaris complex	209964 (4.6)
Other	166834 (3.7)

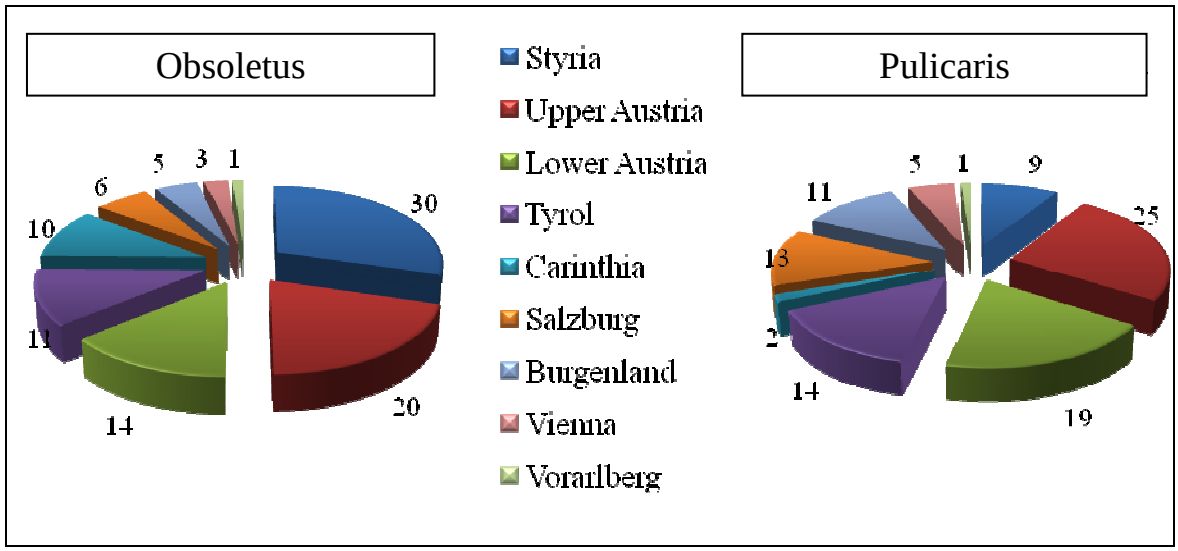
For the creation of the main statistical tables and the distribution maps, only the subclasses Obsoletus and Pulicaris complexes were considered because the class “others” comprises only 3.7% of the total *Culicoides* spp. caught in the Austrian entomological program.

The numbers of species complex by Provinces are very different (Table 5). In Figure 9 the same information can be seen in percentage.

Table 5: Number of *Culicoides* spp., Obsoletus and Pulicaris complexes trapped during all the study period in each Province.

Provinces	***Culicoides* spp.**	**Obsoletus complex**	**Pulicaris complex**
Vorarlberg	60589	51029	2340
Vienna	122619	107311	11310
Burgenland	255924	195777	23554
Salzburg	295061	247544	26429
Carinthia	449210	429592	3412
Tyrol	519157	470612	30188
Lower Austria	643072	581602	40592
Upper Austria	908015	841871	53308
Styria	1285650	1237162	18832

Figure 9: Percentages of midges from the Obsoletus (on the left side) and Pulicaris complexes (on the right side) trapped in each Austrian Province.

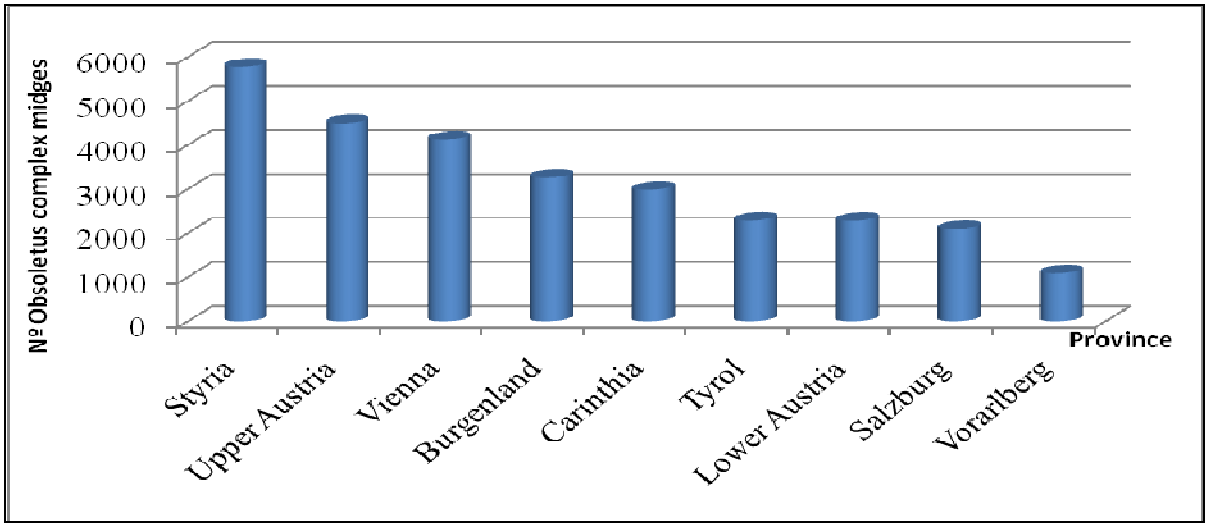


The lack of uniformity has to be considered when interpreting the results. This issue and others, related with data sources and collection will be clarified later in the following chapter.

Given the fact that the number of samples differs by Province, by month and by trap, from now on, only averages of each trap will be used for comparison purpose, and not the absolute number of the vectors.

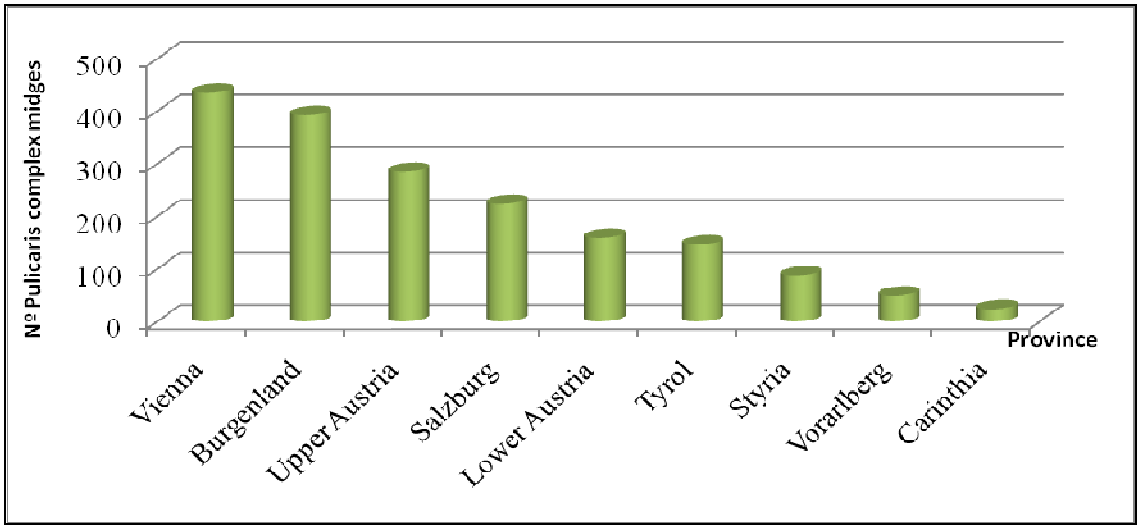
The average of vectors trapped differs between provinces. Styria, Upper Austria and Vienna have an average of more than 4000 Obsoletus by sample (Figure 10).

Figure 10: Average of the amount of vectors from the Obsoletus complex by sample in each province.



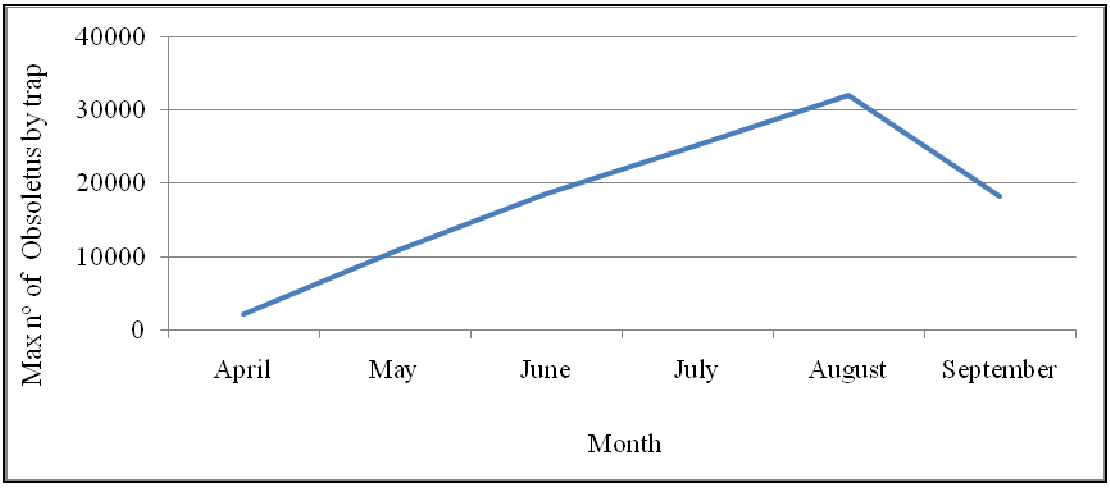
For Pulicaris complex the averages are lower than 500 midges by sample but Vienna and Upper Austria still recorded the bigger averages, together with Burgenland (Figure 11).

Figure 11: Average of the number of vectors from the Pulicaris complex by sample, in each province.



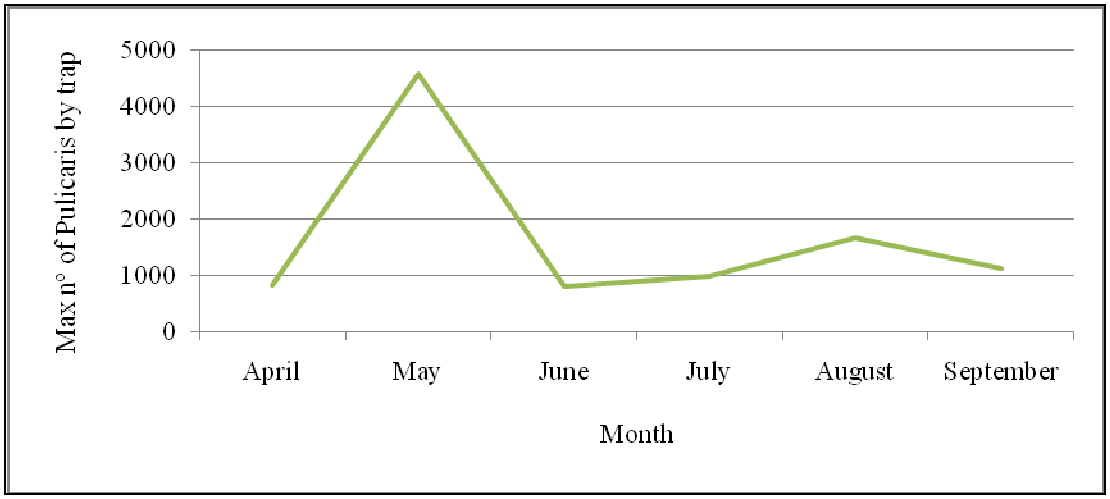
There is a constant rise in the maximum value of Obsoletus complex midges since the beginning of the study period until August, the peak, where the maximum is close to 30 000 (Figure 12). In September the maximum of the amount of midges trapped starts to decrease.

Figure 12: Evolution of the maximum value by month along the study period for Obsoletus complex.



For Pulicaris complex the evolution is irregular with a peak in May, corresponding to 4500, and with values lower than 2000 since June (Figure 13). Nevertheless, there is a second peak in August followed by another decrease in the maximum for September.

Figure 13: Evolution of the maximum value by month along the study period for Pulicaris complex.



5.2 **Statistical modelling analysis**

5.2.1.1 Monthly analysis

During the statistical analysis, 10 of the 16 variables were significant, 8 for the Obsoletus complex, 9 for the Pulicaris complex and 7 for both complexes (Table 6).

Table 6: Significant variables from April to September.

Variables	Obsoletus complex	Pulicaris complex
Avg_tmin	ns	ns
Avg_tmean	ns	ns
Avg_tmax	June	July
Min_tmin	ns	ns
Max_tmax	May, June and September	April
RH_min	ns	ns
RH_avg	ns	ns
RH_max	June and September	June and August
Precipitation	April and July	April, July and August
Days_rain	April, August and September	ns
Wind_avg	ns	June
Wind_max	May	June
Animal density	September	June and September
Altitude	April, June and August	April and August
Land cover	ns	ns
NDVI	ns	June, July and September

Non significant–ns (with $p>0.05$)

During the statistical modelling process 6 listed variables, weren't significant for both complexes: Avg_tmin, Avg_tmean, Min_tmin, RH_min, RH_avg and Land cover. Only 3 variables weren't significant for a particular complex, Wind_avg and NDVI weren't significant for Obsoletus complex and Days_rain for Pulicaris complex.

The outputs of the statistical program R for the backward selection procedure during the model reduction, and the parameter estimates are shown for each month from Table 7 to Table 12.

In April (Table 7) the precipitation and the altitude played the same negative influence on the amount of vectors trapped for both complexes. With observed positive influence there were the days with rain for Obsoletus complex and the absolute maximum of the maximal temperature for the Pulicaris complex.

Table 7: R outputs with the significant variables for April for each complex.

April			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	4.505	2.48e-4
	Precip	-0.274	0.030
	Days_rain	0.202	0.024
	Altitude	-0.005	3.75e-05
Pulicaris	Intercept	-8.231	0.067
	Precip	-0.221	0.005
	Max_tmax	0.547	0.005
	Altitude	-0.003	0.003

Estimate (regression parameters) and p value (significance)

In May (Table 8), for the Obsoletus complex, the maximum wind speed started to have a negative influence in contrast with the positive effects of the absolute maximum of the maximal temperatures. In this month there were no significant variables for the Pulicaris complex.

Table 8: R outputs with the significant variables for May for each complex.

May			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	-3.895	0.421
	Max_tmax	0.440	0.005
	Wind_max	-0.022	0.016
Pulicaris	No significant variables found		

Estimate (regression parameters) and p value (significance)

In June (Table 9) only the maximal relative humidity had influence for both complexes, positive and negative for the Obsoletus and Pulicaris complexes respectively. For the Obsoletus complex the altitude and the average of the maximum temperatures had a positive

effect on the amount of vectors trapped. The absolute maximum temperatures played a negative role. For the Pulicaris complex, the wind started to influence with a positive role played by the maximum wind speed in contrast with the average wind speed. The NDVI plays a negative influence in contrast with the animal density.

Table 9: R outputs with the significant variables for June for each complex.

June			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	-3.212	0.749
	RH_max	0.132	0.043
	Max_tmax	-0.910	0.008
	Altitude	0.003	0.06
	Avg_tmax	1.112	0.011
Pulicaris	Intercept	44.696	0.001
	Animal_density	0.013	0.036
	RH_max	-0.166	0.004
	NDVI	-0.121	0.032
	Wind_avg	-2.284	1.86e-4
	Wind_max	0.018	0.010

Estimate (regression parameters) and p value (significance)

For July (Table 10), all the significant variables had a negative influence. Only the precipitation is significant for the two complexes and is the only variable found for the Obsoletus complex. The NDVI and the average of the maximum temperatures are the other variables that influenced the amount of vectors trapped from the Pulicaris complex.

Table 10: R outputs with the significant variables for July for each complex.

July			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	8.902	1.48e-15
	Precip	-0.180	0.05

Table 10: R outputs with the significant variables for July for each complex (continuation).

Pulicaris	Intercept	37.318	4.3e-4
	Precip	-0.244	0.004
	Avg_tmax	-0.485	0.015
	NDVI	-0.093	0.006

Estimate (regression parameters) and p value (significance)

In August (Table 11) the altitude and the rain played a role in both complexes. The altitude had a positive influence on both complexes in contrast with the days with rain for the Obsoletus complex. For the Pulicaris complex the precipitation and the maximum of the relative humidity had a negative role in the amount of vectors trapped.

Table 11: R outputs with the significant variables for August for each complex.

August			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	8.497	2.66e-10
	Days_rain	-0.184	0.033
	Altitude	0.003	0.014
Pulicaris	Intercept	17.403	1.43e-4
	Precip	-0.199	0.015
	HR_max	-0.147	0.003
	Altitude	0.003	8.36e-4

Estimate (regression parameters) and p value (significance)

In September (Table 12) the animal density had a positive influence on both complexes. The days with rain remained with a negative influence for the Obsoletus complex together with the maximum of the relative humidity. The maximum temperatures had a positive influence in contrast with June, and in accordance with May. For the Pulicaris complex, the NDVI had a negative influence in accordance with what happened in the months before.

Table 12: R outputs with the significant variables for September for each complex.

September			
Complex	Variable	Estimate	p value
Obsoletus	Intercept	8.771	0.173
	Days_rain	-0.193	0.024
	Animal_density	0.016	0.022
	RH_max	-0.182	0.004
	Max_tmax	0.534	0.005
Pulicaris	Intercept	21.993	1.28e-4
	Animal_density	0.018	0.024
	NDVI	-0.094	9.58e-4

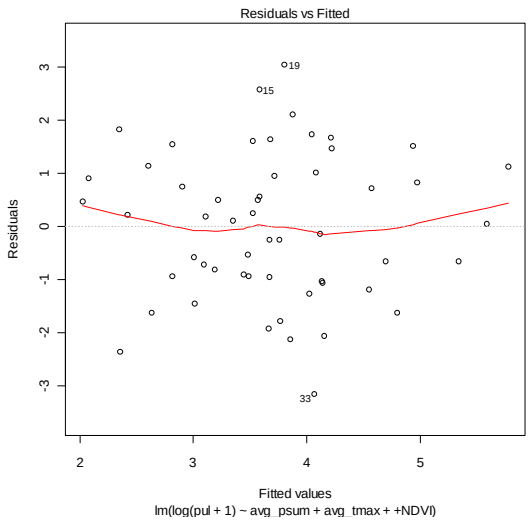
Estimate (regression parameters) and p value (significance)

The R adjusted was lower than 45% for all the models. Even though some of the models covered less than 20% of the explanation on the variability of the vectors trapped, their risk maps were also represented in the section 5.3.

The assumptions of independence, linearity, constant variance, normality, and absence of outliers were all carefully examined and approximately met.

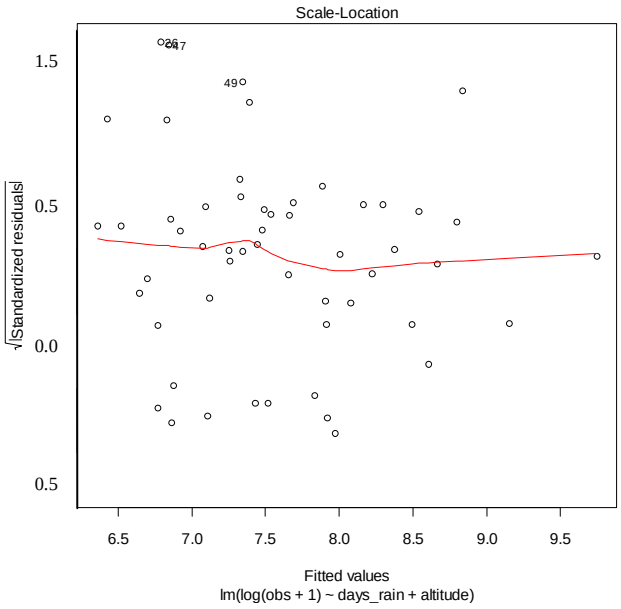
Independence between observations was evaluated based on the information of the design and data collection procedure. The assumption of linearity was checked (Figure 14) by plotting the residuals of the model against the fitted values. The line doesn't have a clear trend and is approximately close to a horizontal line around 0.

Figure 14: Examples of a plot to check for linearity where the red line is close to a X=0 line.



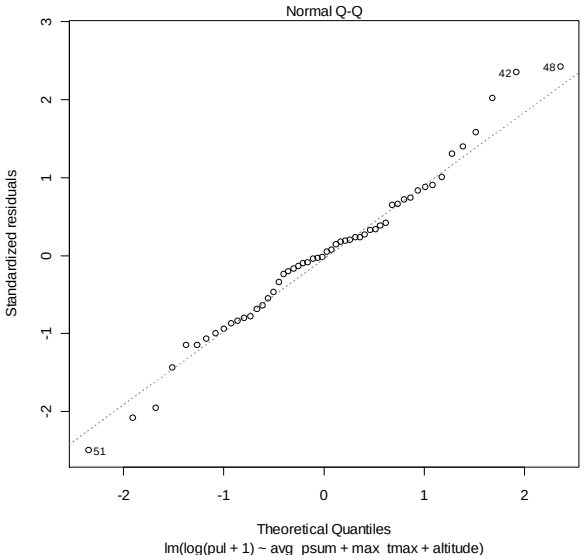
The assumption of homocedasticity was evaluated based on plots with the standard deviation of residuals against the fitted values. In Figure 15 more values are close to a horizontal line meaning approximately equal variance.

Figure 15: Examples of a plot to check for homocedasticity where the values are approximately close to a horizontal line.



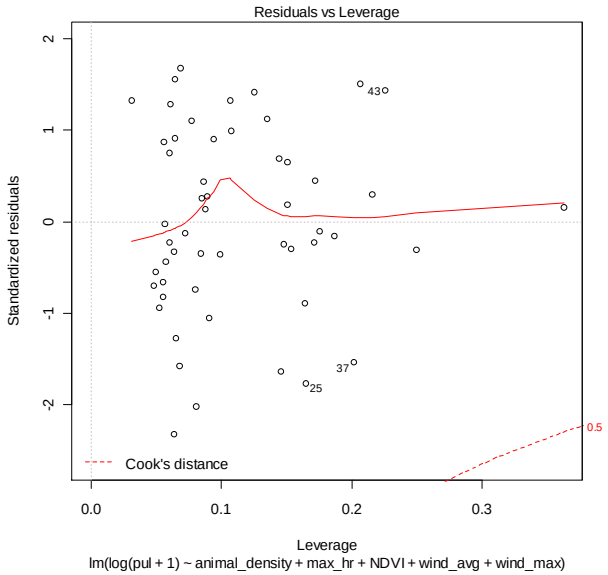
Probability plots were used to check for normality after a log transformation (Figure 16).

Figure 16: Example of one of the QQ plots showing how close the residuals (represented by the points) fit a normal distribution (represented by the line in the graph).



No outliers were found with the method of Cook's distance (Figure 17) since no observation was substantially larger than 0.5 or other Cook's distance line.

Figure 17: Example of a plot to check for outliers showing that there are no points that lay outside the Cook's distance line.



5.2.1.2 Seasons analysis

The seasons covered by the study period had 9 of the 16 variables as significant, 6 for each complex and within those 3 matches for both complexes (Table 13).

Table 13: Significant variables for the spring and summer analysis.

Variables	Obsoletus complex	Pulicaris complex
Avg_tmin	ns	ns
Avg_tmean	Summer	ns
Avg_tmax	Spring	ns
Min_tmin	Summer	Spring
Max_tmax	ns	Spring
RH_min	ns	ns
RH_avg	ns	ns
RH_max	ns	Spring and Summer
Precipitation	ns	ns

Table 13: Significant variables for the spring and summer (continuation)

Days_rain	**ns**	**ns**
Wind_avg	Spring	ns
Wind_max	ns	ns
Animal density	ns	Summer
Altitude	Summer	Summer
Land cover	ns	ns
NDVI	Spring	Summer

During the statistical modelling process for the seasons, 7 variables weren't significant for both complex (Avg_tmin, RH_min, RH_avg, Precipitation, Days_rain, Wind_max and Land cover). The Animal density RH_max Max_tmax weren't significant for Obsoletus complex and Avg_tmean, Avg_tmax and Wind_avg for Pulicaris complexes. The Min_tmin, Altitude and NDVI were significant for both complexes adding the fact that the altitude was significant in the same season for both complexes.

The outputs of the statistical program R for the backward selection procedure during the model reduction, and the parameter estimates are shown for each season in Table 14 and 15.

Table 14: R outputs with the significant variables for the Obsoletus complex for each season.

Obsoletus Complex			
Season	Variable	Estimate	p value
Spring	Intercept	-8.918	5.86e-4
	Avg_tmax	0.317	7.75e-08
	NDVI	0.047	0.006
	Wind_avg	0.040	0.054
Summer	Intercept	-0.771	0.700
	Min_tmin	-0.2	0.049
	Avg_tmed	0.463	0.001
	Altitude	0.002	0.001

Estimate (regression parameters) and p value (significance)

For the Obsoletus complex (Table 14) there was a contrast in the influence of the temperature in the spring and in the summer. It was observed a positive influence of the maximum temperatures in the spring and the mean temperatures in the summer. In the former, there is

also a positive influence of the altitude. The NDVI and the average of the wind speed played a positive role in the amount of vectors trapped in the spring and the minimum temperatures had a negative influence in the summer.

Table 15: R outputs with the significant variables for the Pulicaris complex for each season.

Pulicaris complex			
Season	Variable	Estimate	p value
Spring	Intercept	4.806	0.104
	RH_max	-0.068	0.04
	Max_tmax	0.149	0.017
	Min_tmin	0.159	0.019
Summer	Intercept	30.928	3.53e-10
	Animal_density	0.014	0.003
	RH_max	-0.106	0.001
	NDVI	-0.092	1.44e-06
	Altitude	0.002	0.001

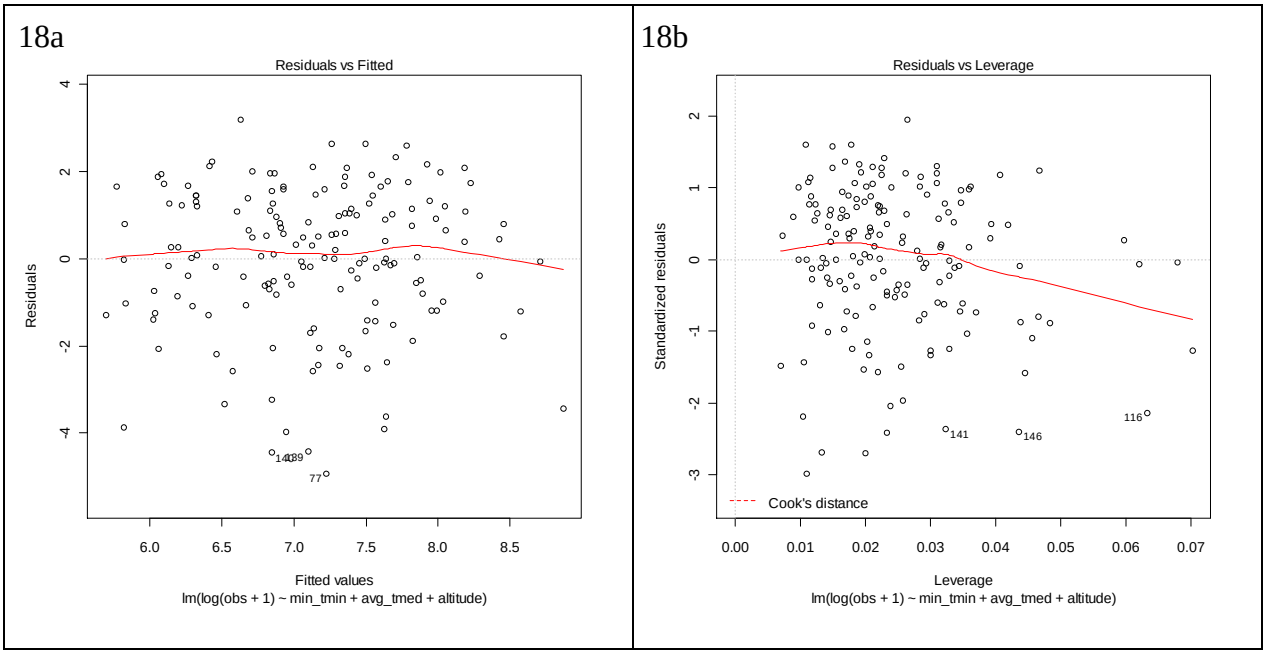
Estimate (regression parameters) and p value (significance).

For the Pulicaris complex (Table 15) in the spring the extremes in the temperatures had a positive role in the amount of vectors trapped. The Maximum of the relative humidity played a negative influence in both seasons. As in the Obsoletus complex, the altitude also had a positive influence in the summer. In the previous, the animal density had a positive role in contrast with the NDVI.

The R adjusted was lower than 52% for all the models. Even though some of the models covered less than 20% of the explanation on the variability of the vectors trapped, their risk maps were also represented.

The model assumptions were all carefully checked and approximately met, in the same way as in the section before (5.2.1.1). In Figure 18 there are plots for checking linearity (18a) and outliers (18b).

Figure 18: Examples of plots to check for linearity (18a) and outliers (18b).



5.3 Risk maps

This section will be discussed in terms of the expected averages of the amount of midges trapped by month (5.3.1) or season (5.3.2) for each complex.

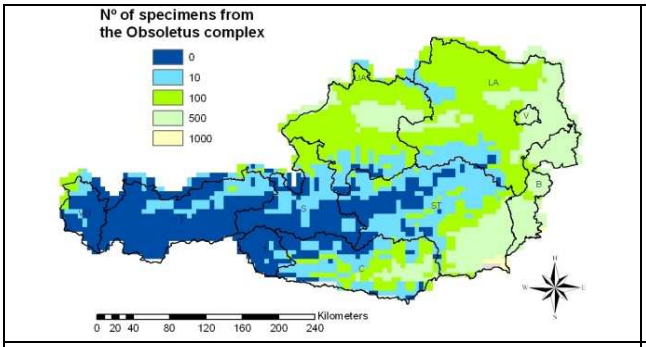
The risk maps for the Obsoletus and Pulicaris complexes are presented from Figure 19 to 24 and from Figure 25 to 29 for each month and season respectively, followed by the maps with the significant variables and a detailed analysis.

5.3.1 Monthly analysis

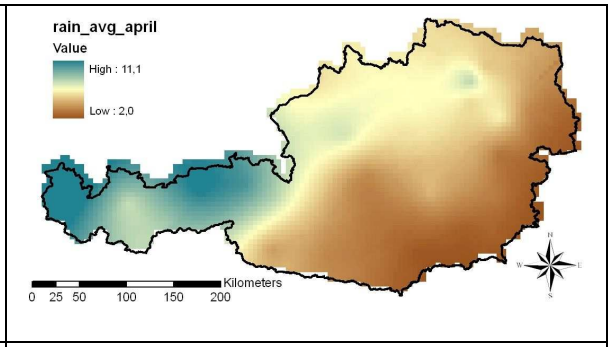
5.3.1.1 Monthly risk maps for Obsoletus complex

The risk maps for the Obsoletus complex are presented next, followed by the maps for the significant variables in each month.

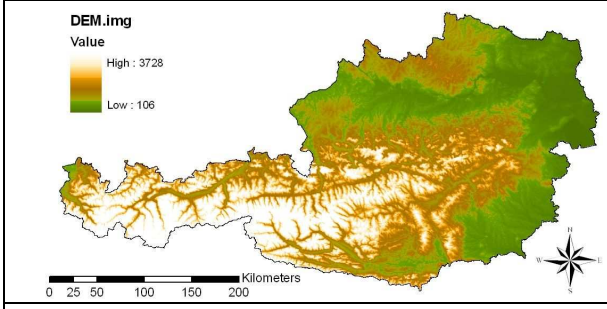
Figure 19: Risk maps for April with the expected amount of midges trapped from the Obsoletus complex (19a), and the respective significant variables (19b-d).



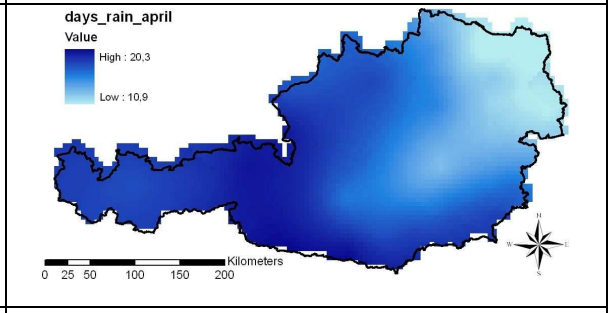
19a



19b



19c



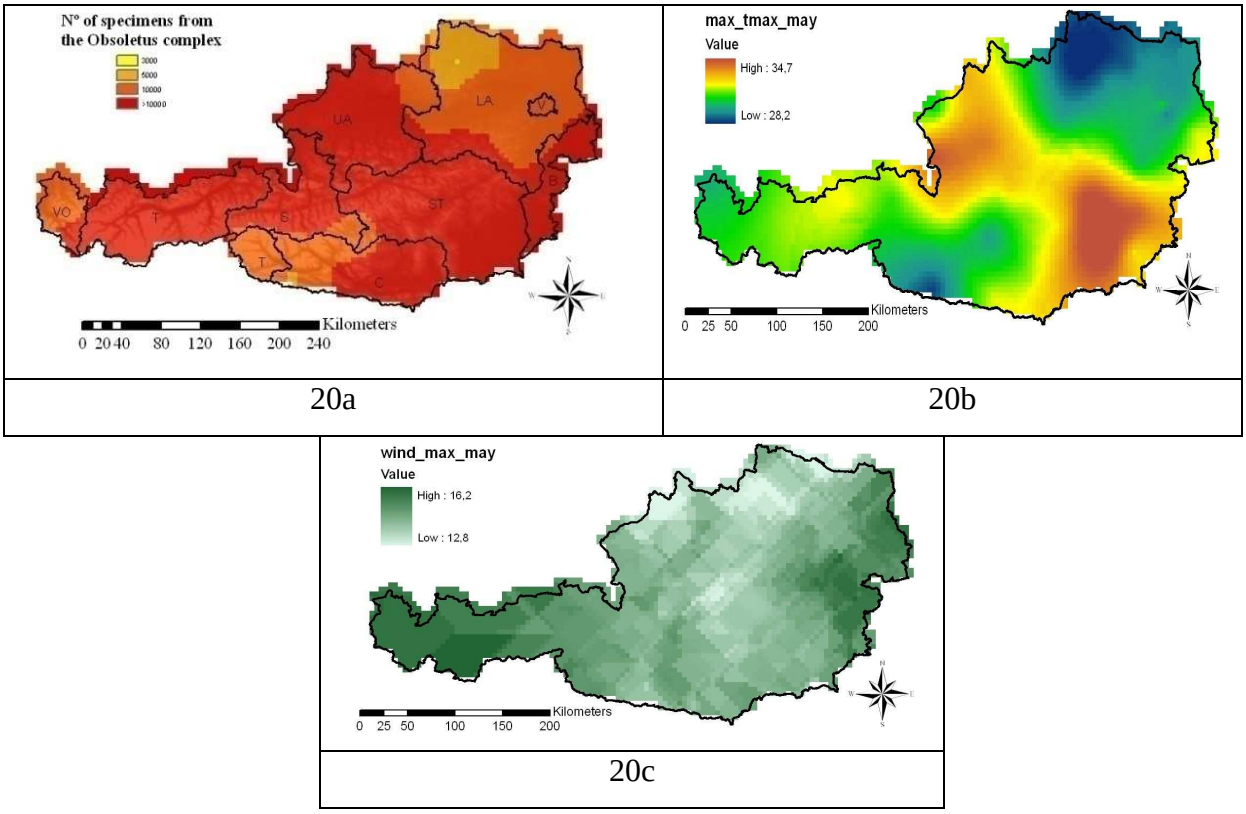
19d

In April (Figure 19a) only a small area in the South Eastern part of Styria is expected to have between 500-1000 midges trapped. The expected values for the whole country in the maps match those of the entomological surveillance because this present month had the lowest maximum value of midges recorded during the study period and the Provinces with the lowest

catches were Vorarlberg, Salzburg, Lower Austria and Tyrol. In the previous provinces in general, no midges, or less than 10 are expected to be captured.

It is expected that in areas with less precipitation (Figure 19b) and lower altitudes (Figure 19c) record bigger amounts of Obsoletus complex midges. For the days with rain (Figure 19d) the relation is less clear. Even though there seems to be a negative relation between this variable and the amount of midges captured, which is, less vectors are expected to be trapped when there are more days with rain in the month, the statistical analysis revealed a small positive relation between them.

Figure 20: Risk maps for May with the expected amount of midges trapped from the Obsoletus complex (20a), and the respective significant variables (20b-c).



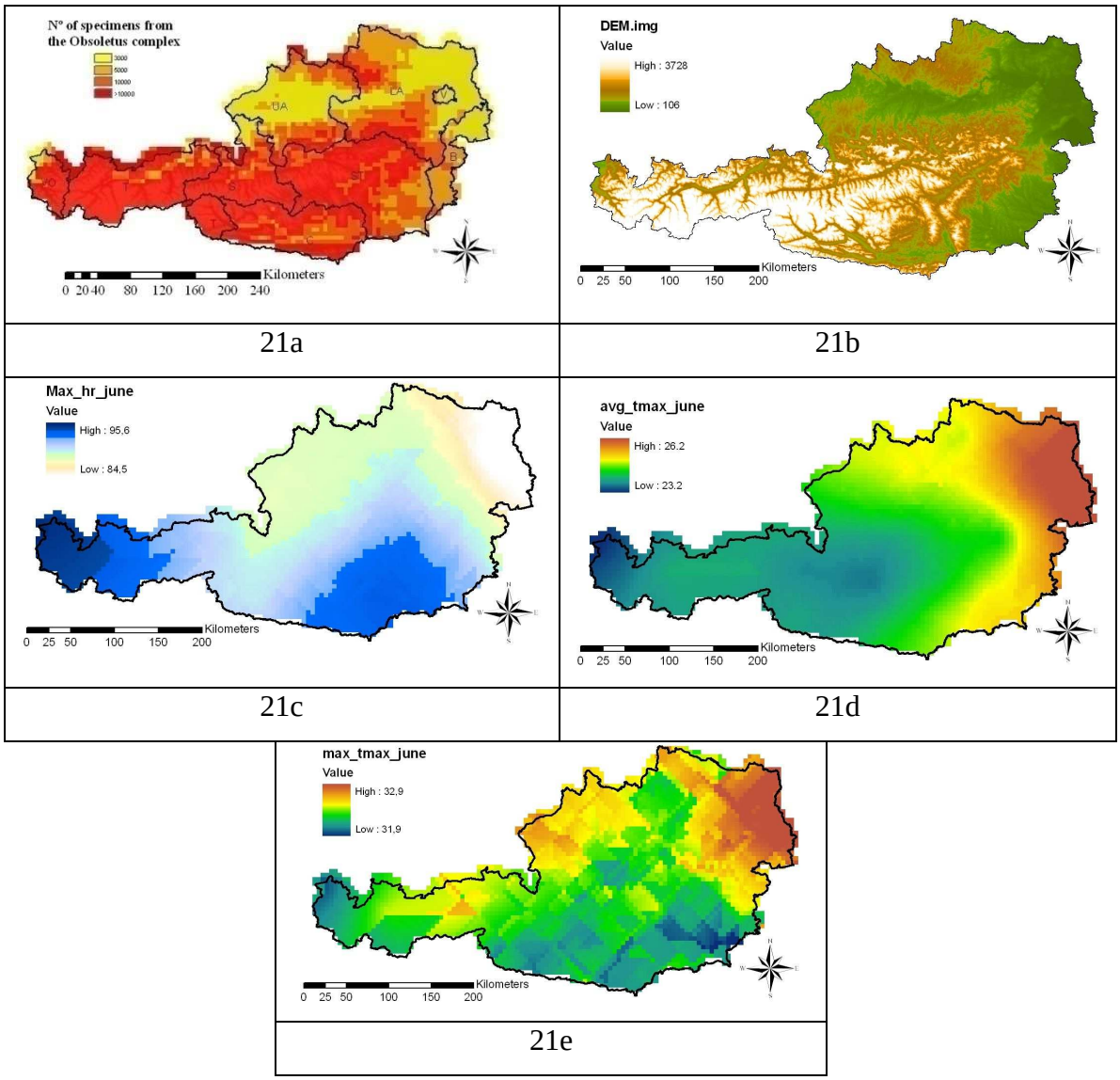
20a | 20b

20c

In May (Figure 20a), there is a complete different scenario as the expected values range from 2000 to more than 10 000. In general, all of the country expects more than 5000 midges to be captured, except for a Northern area in Upper Austria in May.

Considering the significant variables for this month it is expected a positive relation between the areas with bigger maximum temperatures (Figure 20b) and less wind speeds (Figure 20c), which is, with higher amounts of midges trapped.

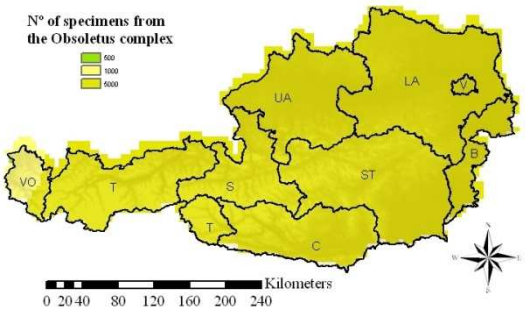
Figure 21: Risk maps for June with the expected amount of midges trapped from the Obsoletus complex (21a), and the respective significant variables (21b-e).



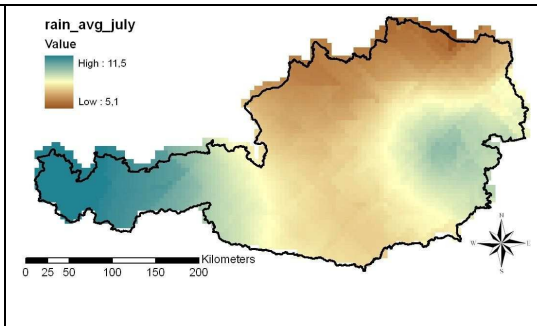
In June (Figure 21a), the situation is similar with the previous month, the expected values range from 2000 to more than 10 000. In general, all of the country expects more than 5000 midges to be captured, except for Lower Austria and Burgenland.

It is also expected that areas with bigger amounts of vectors trapped have higher: altitude (Figure 21b), relative humidity (figure 21c) and average maximum temperatures (figure 21d). In contrast, in Figure 21e it is expected that, in areas with bigger absolute maximum temperatures, there are lower amounts of midges captured.

Figure 22: Risk maps for July with the expected amount of midges trapped from the Obsoletus complex (22a), and the respective significant variable (22b).



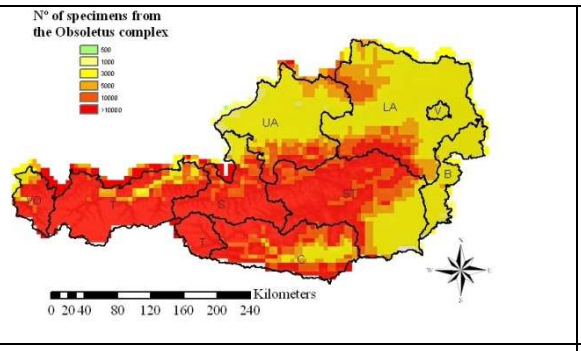
22a



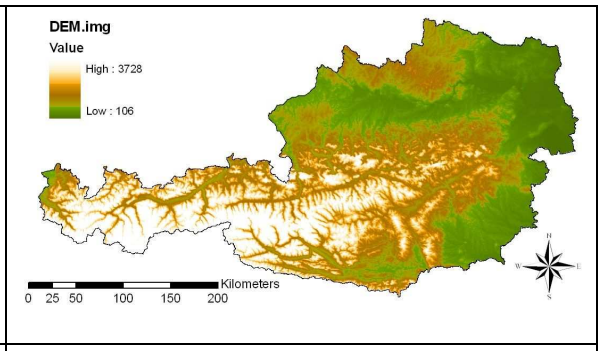
22b

In July, there is a difference in the risk map when compared with the previous and the following month. Since the environmental variables did not vary to a great extent, the explanation might be in other variables not taken in consideration the problems with the data. Also, the expected negative relation between the abundance of vectors trapped and the precipitation (Figure 22b) is less clear. Thus, this month won't be included in the analysis.

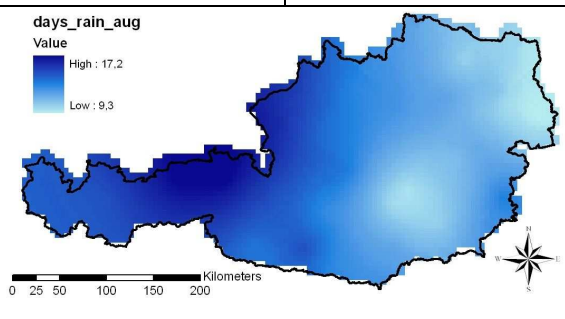
Figure 23: Risk maps for August with the expected amount of midges trapped from the Obsoletus complex (23a), and the respective significant variables (23b-c).



23a



23b

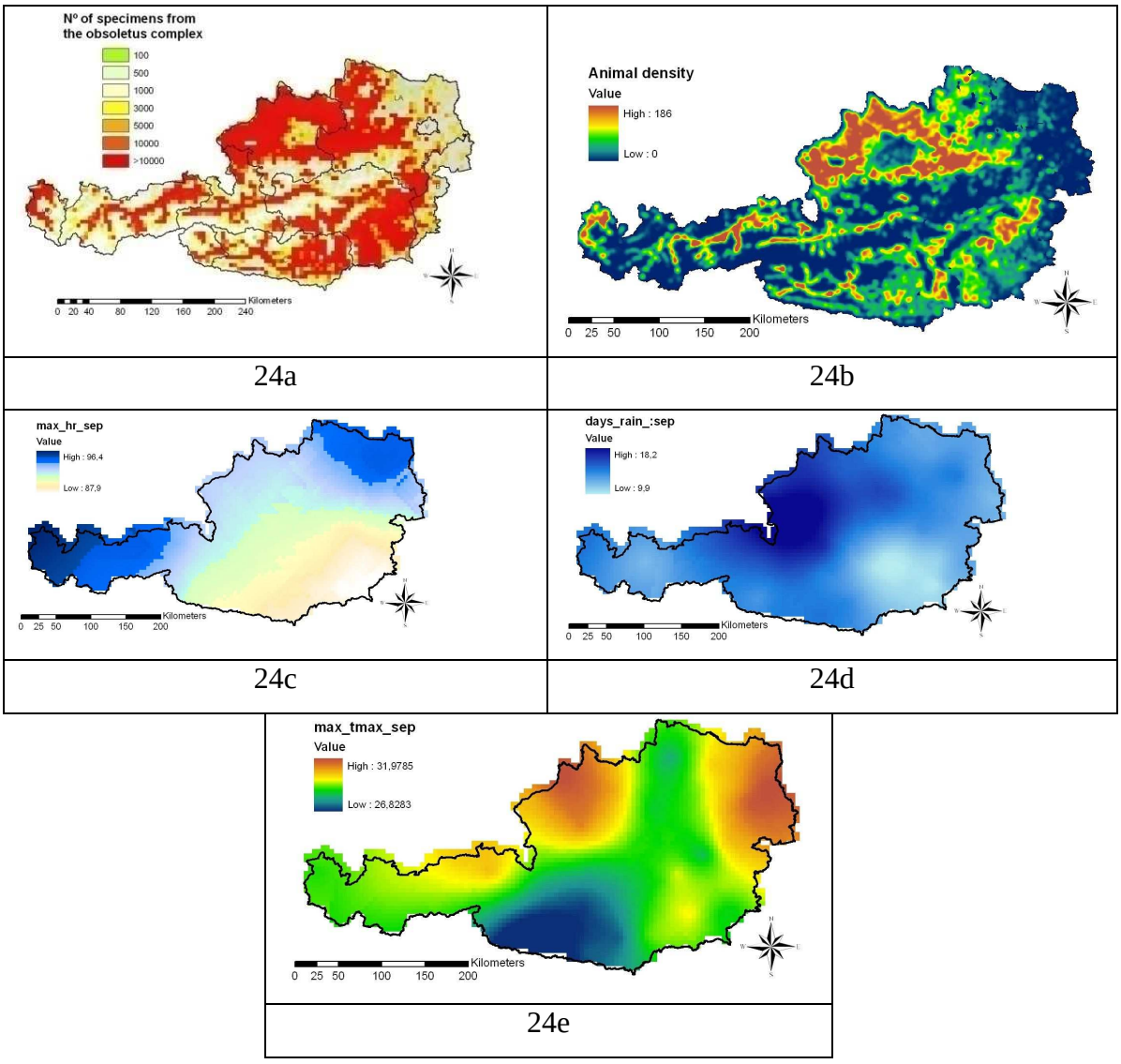


23c

From May to August, the expected amount is higher than in the rest of the study period.

In August (figure 23a), a massive amount of vectors is also expected but the more Eastern and Northern Provinces are expected to have less than 3000 midges. There is a clear positive relation between the altitude (Figure 23b) and the amount of Obsoletus complex midges trapped, which, the bigger the altitude, the less amount of vectors trapped is expected. In Figure 23c the relation is less clear, however the statistical analysis revealed a positive relation between the precipitation and the abundance of the vectors captured.

Figure 24: Risk maps for September with the expected amount of midges trapped from the Obsoletus complex (24a), and the respective significant variables (24b-e).



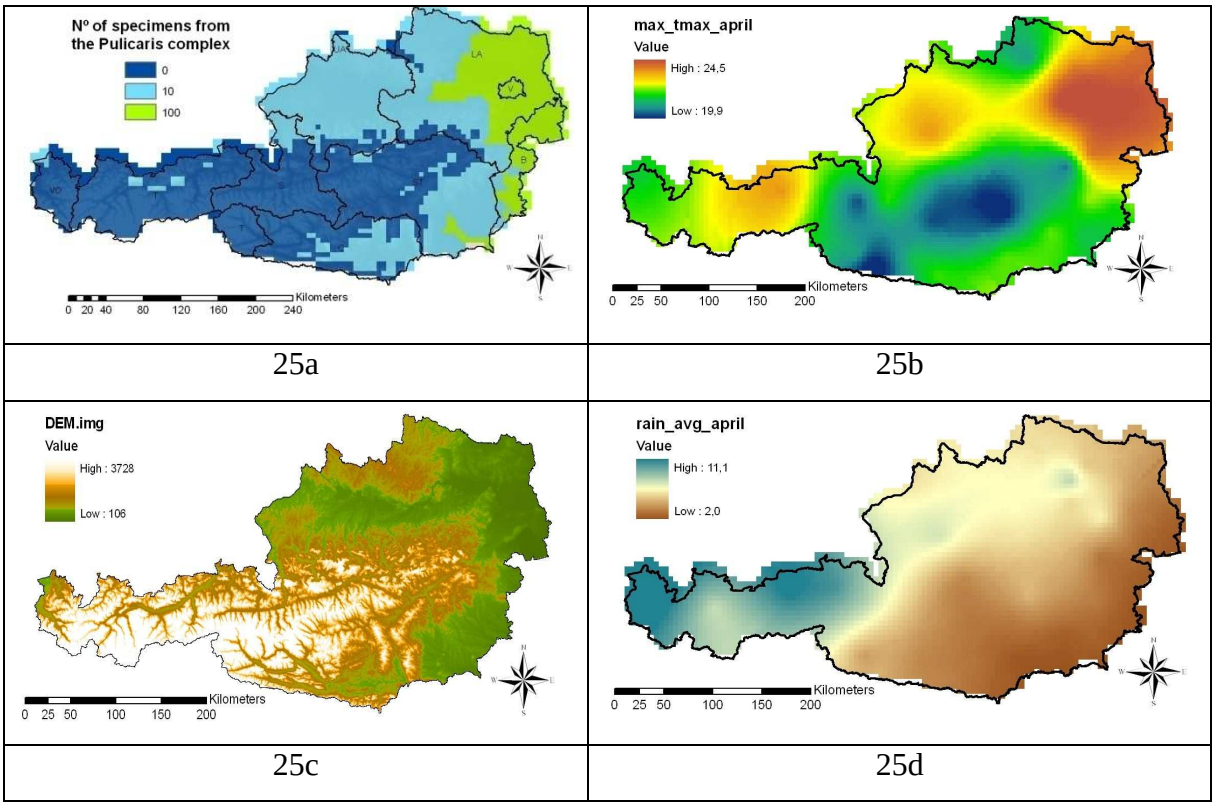
In September (Figure 24a), there is a huge difference between and within Provinces. The amount of vectors expected ranges approximately from 10 to more than 10 000. Microclimate conditions might explain this discrepancy between neighboring regions. Nevertheless, the further most Eastern Austrian areas continue to have likely fewer amounts of vectors.

It is expected that areas with higher animal density (Figure 24b) record bigger amounts of vectors trapped, in contrast with the maximum relative humidity (Figure 24c). For the days with rain (Figure 24d) and the absolute maximum temperature (Figure 24e) the expected relation is less clear but from the statistical analysis it was demonstrated that there is a positive relation for the first variable in contrast with the last one.

5.3.1.2 Monthly risk maps for Pulicaris complex

Following the previous section order, the risk maps for the Pulicaris complex are presented before the maps with the presentation of the significant variables in each month.

Figure 25: Risk maps for April with the expected amount of midges trapped from the Pulicaris complex (25a), and the respective significant variables (25b-d).



25a | 25b

25c | 25d

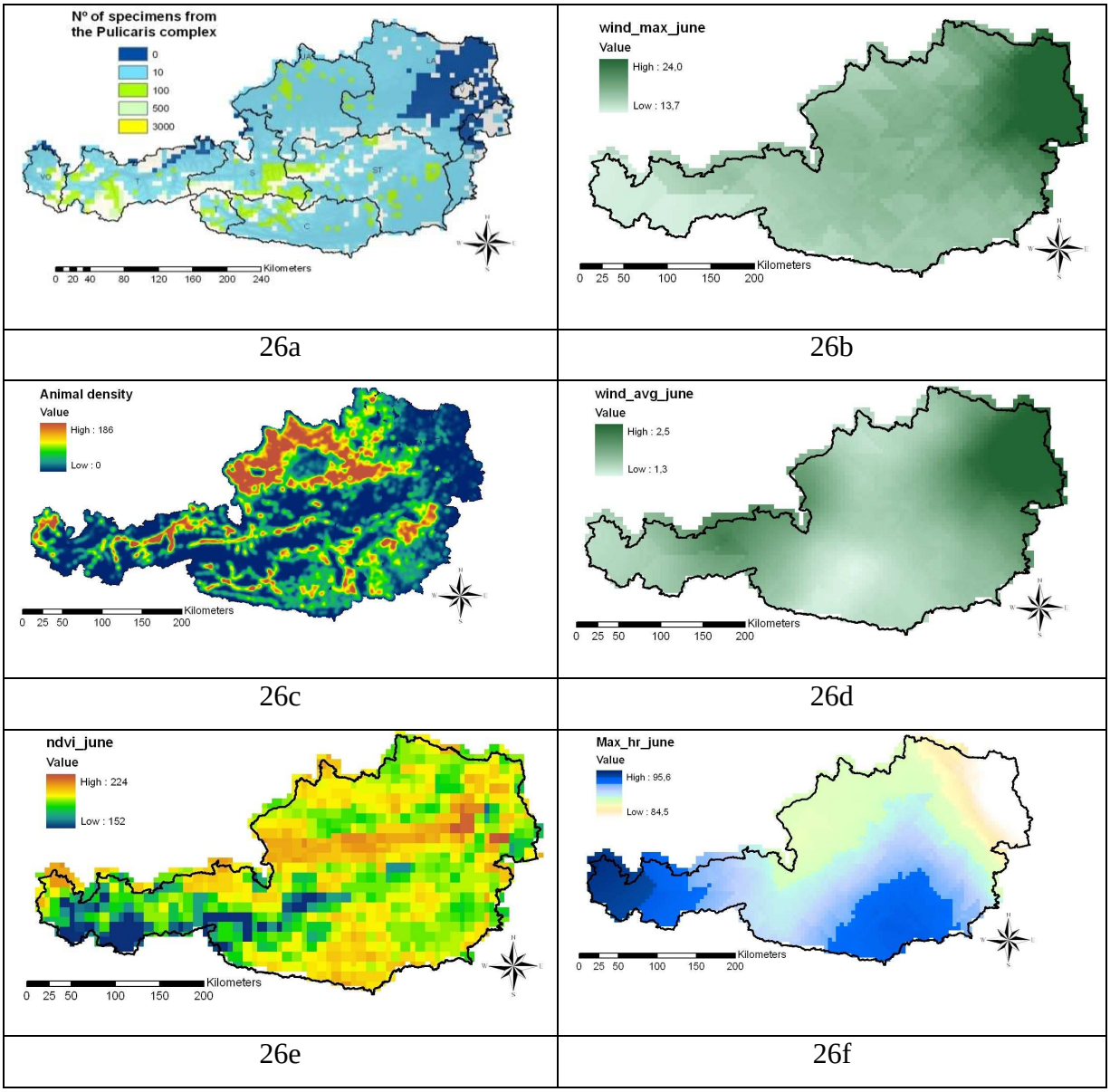
When compared with the other complex in study, only April (Figure 25a) is quite similar. The further most Eastern parts of the country (Burgenland, Vienna and some of the Lower Austria) are expected to have between 10-100 midges. The remaining provinces expect between 0-10 midges.

From Figure 25b it is expected a clear positive relation between the absolute maximum temperatures and the amount of vectors trapped, especially for the Eastern areas of the

country. In contrast, in areas with increasing altitude (Figure 25c) and precipitation (Figure 25d) the expected abundance of midges captured decreases.

In May, the results of the entomological data revealed a peak in the amount of vectors trapped. Unfortunately, no variables were significant for this month, thus, no risk map could be created.

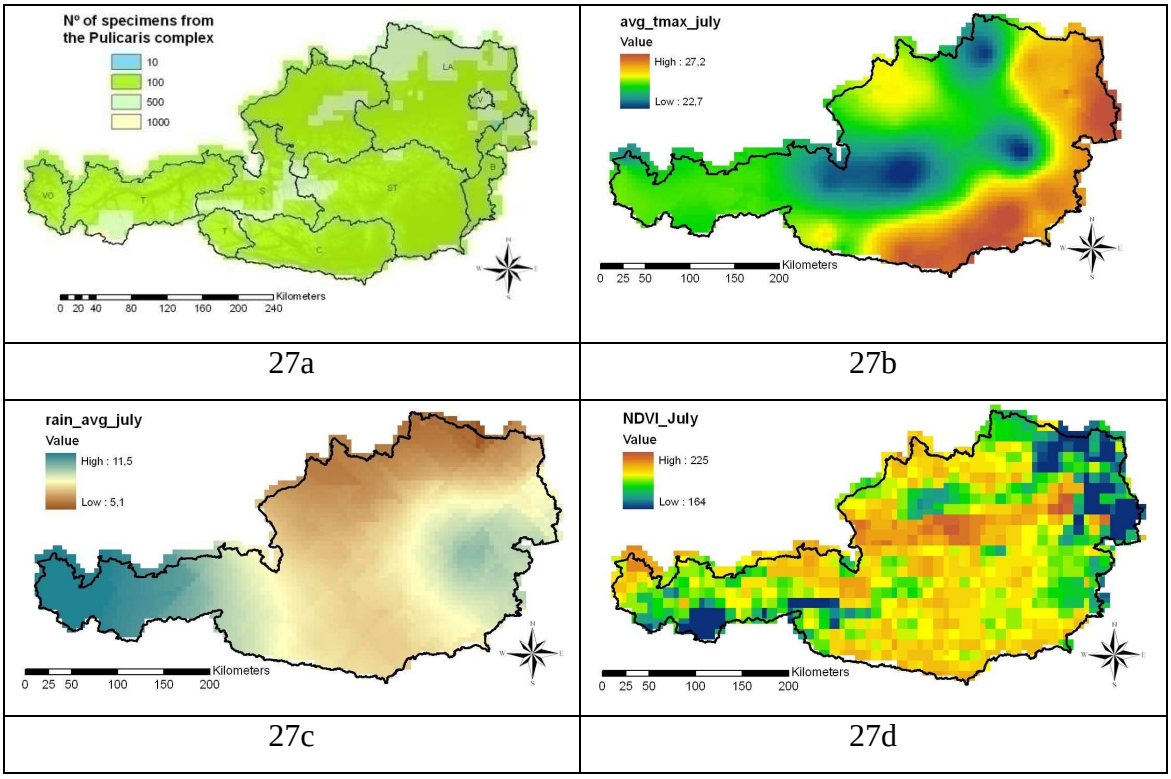
Figure 26: Risk maps for June with the expected amount of midges trapped from the Pulicaris complex (26a), and the respective significant variables (26b-f).



When compared with April, there is a complete difference in the distribution of the amount of catches in June (Figure 26a). The Northern eastern regions are likely to have no *Culicoides* captured and the remaining parts of the country, almost 80%, expect from 10-500.

In areas with higher absolute maximum wind speeds (Figure 26b) and animal density (Figure 26c) it is expected that the amount of vectors trapped increases. Less clear is the negative relation between the previous with the average wind speed (Figure 26d), the NDVI (Figure 26e) and the maximum of the relative humidity (Figure 26f).

Figure 27: Risk maps for July with the expected amount of midges trapped from the Pulicaris complex (27a), and the respective significant variables (27b-d).



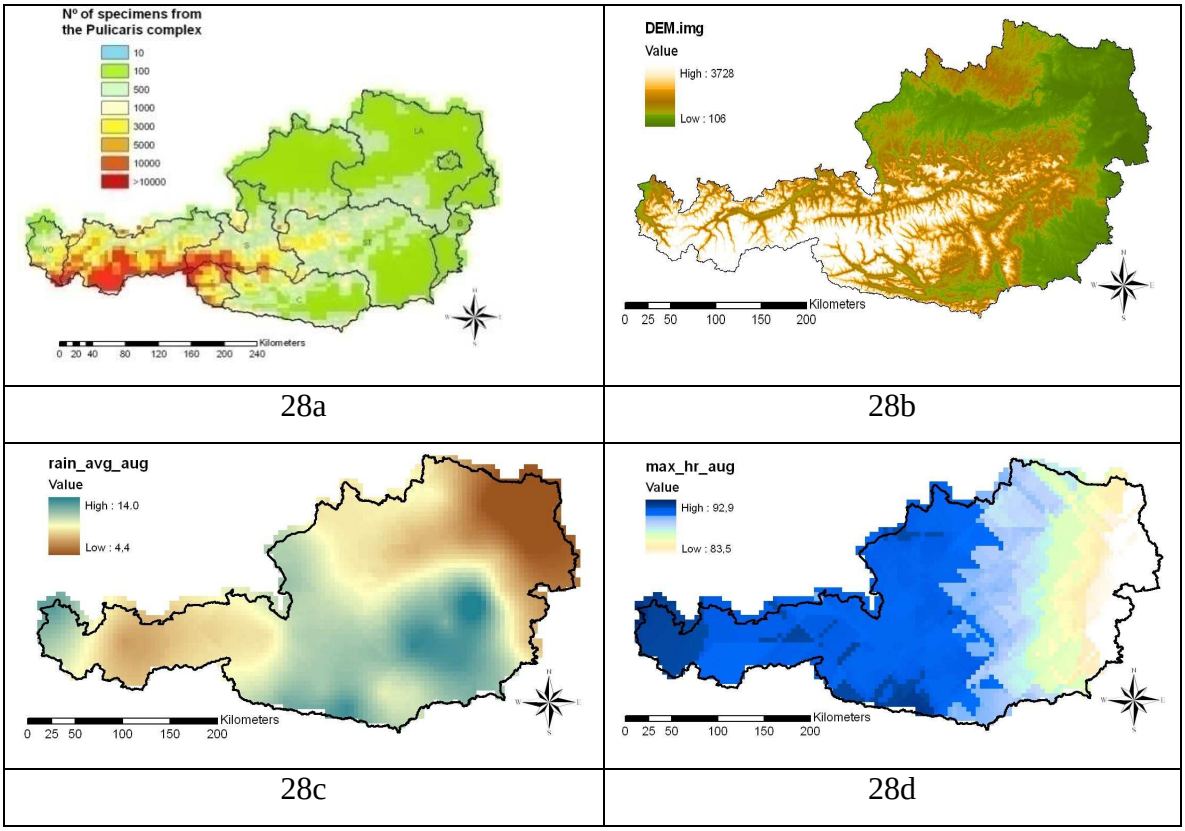
27a | 27b

27c | 27d

In July there is a homogenous distribution of the amount of vectors, similar with what happened for the previous complex. Still, for Pulicaris, according with the results from the surveillance, there is a small increase in the amount of vectors trapped from the beginning of July until August, when there was a second peak, much lower than the one in May, and starts decreasing until the end of September. The maps agree with this pattern.

The expected negative relation between all the significant variables: average of the maximum temperatures (Figure 27b), precipitation (Figure 27c) and NDVI (Figure 27d) is less clear, when compared with previous months. Southeastern areas recorded higher maximum temperatures and the amount of vectors expected is lower than 100. The same happens for the precipitation, where the further most east and west areas of the country recorded higher volume of precipitation together with lower amounts of Pulicaris complex midges.

Figure 28: Risk maps for August with the expected amount of midges trapped from the Pulicaris complex (28a), and the respective significant variables (28b-d).



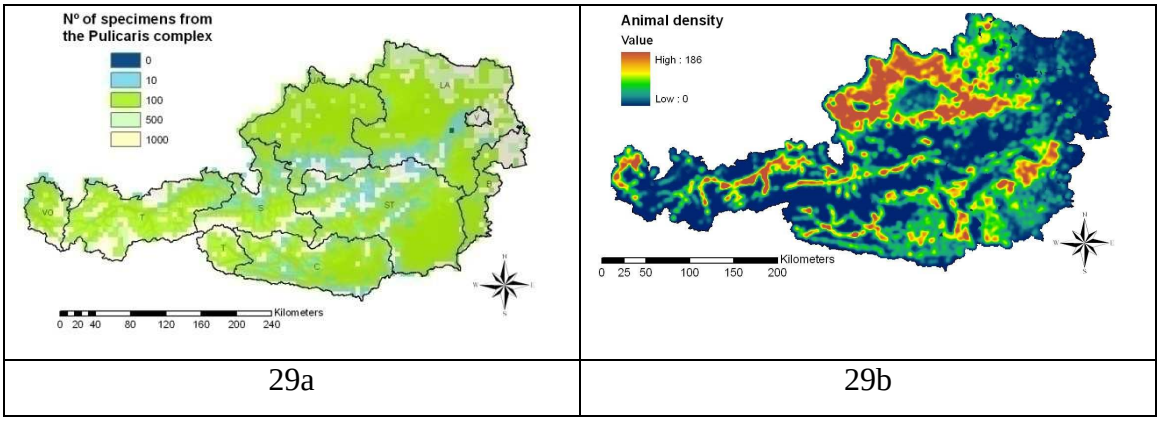
28a | 28b

28c | 28d

In August (Figure 28a), mainly in the Southern areas of Tyrol are expected more than 10000 *Culicoides*. This value decreases in the neighboring regions. The Eastern and Northern Provinces are expected to have in general captures close to 100.

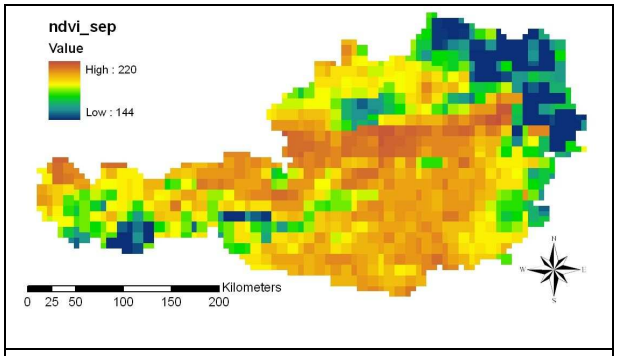
The number of vectors trapped is augmented with the increase in the altitude (Figure 28b) and decrease in both precipitation (Figure 28c) and relative humidity (Figure 28d).

Figure 29: Risk maps for September with the expected amount of midges trapped from the Pulicaris complex (29a), and the respective significant variables (29b-c).



29a | 29b

Figure 29: Risk maps for September with the expected amount of midges trapped from the Pulicaris complex (29a), and the respective significant variables (29b-c) (continuation).



29c

In September (Figure 29a), no more than 1000 *Culicoides* are expected. Usually, the catches are likely to have close to 100 midges.

It is expected an increase in the amount of vectors trapped in areas with higher animal density (Figure 29b) and lower NDVI (Figure 29c).

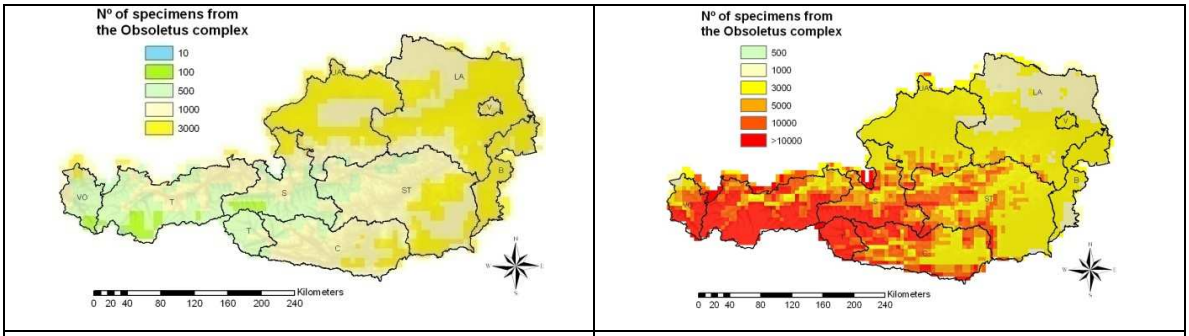
5.3.2 **Season analysis**

Since the season risk maps (Figures 30 and 31) agree with the monthly analysis (5.3.1), the explanation of the results won't include the comparison with the maps of the significant variables, to avoid repeating information.

5.3.2.1 **Season risk map for Obsoletus complex**

In the risk maps Figure 30 it is expected that in the spring the captures of *Culicoides* are in the range of 3000 vectors in the Eastern Provinces and some parts of the Northern Austria. The rest of the country expects less than 1000, mostly between 100 and 500. In the summer there is a complete difference, with the further most Western areas having more than 10000 and the others, except for the North, between 3000.

Figure 30: Risk maps for the Obsoletus complex for each season.



Spring | Summer

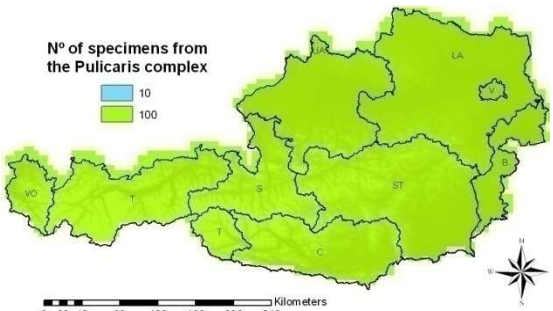
5.3.2.2 Season risk map for Pulicaris complex

As expected, for Pulicaris, the risk maps (Figure 31) show that the expected amounts of midges trapped is much lower for both seasons, when compared with the previous complex.

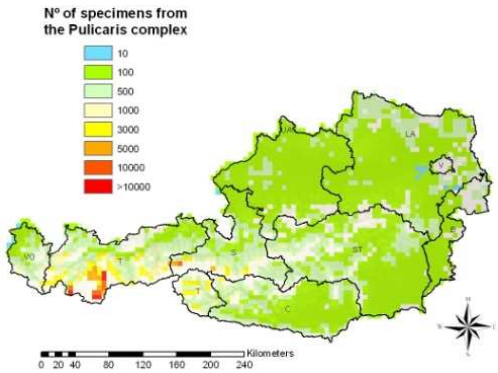
In the spring, more than 100 midges trapped are expected across all the country.

In the summer, in general the amounts expected are not so different from the spring, except that there is a huge variation in some areas, especially in Tyrol, where it can range from 10 to more than 10 000.

Figure 31: Risk maps for the Pulicaris complex for each season.



Spring



Summer

6 DISCUSSION

6.1 Occurrence of Obsoletus and Pulicaris complexes

Specimens of the Obsoletus complex dominated most of the sampling sites. Conversely Pulicaris complex was much less abundant. The results show considerable fluctuations in *Culicoides* abundance and distribution. However, the study only covered part of the activity period. It would be interesting to study the *Culicoides* occurrence during all year to know what is the length of vectors activity period or, furthermore, if there are conditions for overwintering of BTV in Austria. Other interesting criteria that couldn't be included in the study, due to limitations in the data, were the gender and the blood feed status of females.

Together, the two complexes studied represented close to 100% of the *Culicoides* catches. Nevertheless, species outside these complexes might play an important role in the future concerning BTD spread and persistence in Northern Europe.

6.2 Significant variables for the Obsoletus and Pulicaris complexes in Austria in the study period

6.2.1 Monthly analysis

Specific climatic preferences were identified for each month in the two complexes in study.

Variables related with maximal temperatures and humidity were significant for both complexes in the same month together with the precipitation, animal density, altitude and the maximum of the relative humidity.

The precipitation, days with rain, relative humidity and NDVI will be discussed together due to their relation with moist environment.

The precipitation always played a negative role for both complexes. Studies from Meiswinkel and his colleagues (2004b) revealed that rainfall increases generation rates by promoting the availability of breeding sites and increases adult survival rate by avoiding desiccation. However, when the intensity of the rain increases, soils become leached of nutrients and so, unsuitable for breeding sites, together with the inhibition of vectors flight. In this study, the minimum and maximum averages of the rain generally don't justify lixiviation effects (between 2.38 and 19.3 ml). In the same way, the days with rain had a negative influence in the months where it rained more (August and September) and a positive influence when rained less (April). The maximum of the relative humidity had a positive influence in the amount of vectors trapped in the months with less rain in reverse with the months with more

rain for both complexes. In the last there were many days with more than 90% of relative humidity which is unsuitable for the vectors. These three variables highlight the possibility of local inhibition of vectors flight has a more plausible explanation instead of the negative effect on desiccation.

Another variable related with moisture is the NDVI which always had a negative effect in the amount of Pulicaris complex midges trapped. Conversely, in studies from Purse and his partners (2004b), this variable was determinant of the abundance for Pulicaris complex, which indicates that it prefers microclimates with high stable levels of moisture for optimal survival and development. As was mentioned earlier, this is consistent with its breeding site requirements. Nevertheless, *C. pulicaris* ss, favours sparsely to moderately vegetated habitats (lower NDVI) that are open to sunlight (heliophily). Due to the fact that NDVI levels vary between months and had always a negative effect for this complex species, no further conclusions will be drawn from this variable. Nevertheless, future investigation on Austrian vegetation biomass, coverage and productivity, for possible further explanation of this negative effect, is advisable.

The average and maximum of the temperatures increased the amount of vectors trapped in the coldest months (April, May and September) and decreased in the warmer months (June and July) for both complexes. The medium temperatures were already very high in the warmer months, turning the days with the bigger maximums unsuitable for the vectors. Also, the altitude increased the amount of vectors trapped in the warmer months (June and August) and decreased in the cooler month (April) for both complexes. The temperature depends on the altitude and can't, therefore, be separated or interpreted apart from altitude effect. Hilly areas were always cooler compared to areas of lower elevation, even during summer months. (Staubach et al., 2008). In previous entomological studies in Austria from Sehnal and his colleagues (2008), no correlation with altitude could be verified in contrast with this present study. In 2004, Conte and his colleagues referred that *C. obsoletus* are widespread latitudinal and altitudinal across the Paleartic region. Its mean altitudinal range is close to 587.1 m although some specimens were captured at 2100 m in Northern Italy in a study from Purse and his partners (2004b). Even in Austria the altitude ranges from as low as 114 m to Alpine regions peaking at 3798 m, and the Obsoletus complex are found regularly throughout mountainous terrain.

The wind had a different effect between complexes and, within Pulicaris complex, between months. For the Obsoletus complex in May, the increasing of maximum wind speed (ranging from 9.5-21.1 m/s) decreased the amount of vectors trapped. Conversely, for Pulicaris complex, in June, the maximum wind speed (10.9 - 29.9 m/s) had a positive effect together

with a negative effect of the average wind speed (6.2 - 26.5 m/s). Due to the complexity of the wind patterns on vectors flight, caution should be given when interpreting these results. Previous studies from Baylis & Rawlings (1998) have demonstrated that daily *Culicoides* abundance is dependent upon wind speed. Strong wind force obviously has a negative influence on the activity of biting midges due to the vectors inhibition to fly above 11 m/s (Hendrickx, 2008). This explains the negative effect of the wind on the results. On the other hand, wind has also relevance for possible spread scenarios of the vector. The explanation for its positive influence might be on a macroclimate scales, and not local conditions around the trap. In fact, it's possible that new vectors were introduced in the country/region transported in the wind. This might explain the positive influence of the maximum wind speed in June. Nevertheless, this variable and its effect on the vectors should be studied over the time and not at a given point in time. Also, more research over the direction of the winds, in this time period, and its intensity is needed on a national and local scale for further explanation. Studies from Sibermayr (2009), in Austria, also excluded the wind influence to avoid confusion when interpreting results concerning *Culicoides* abundance.

The animal density always increased the amount of vectors trapped for both complexes. *Culicoides* need to feed upon hosts, thus, suitable places for this vectors include areas with high animal density. This variable was obtained gathering the numbers from cattle, sheep and goats because, to date, there is no clear evidence that *Culicoides* of some specie/complex have more affinity to some species of hosts. Even though the value for this variable was the same between months, its inclusion was considered in order to observe if different animal densities between regions, had some effect on the vectors occurrence and amount.

6.2.2 **Season analysis**

Due to the fact that the number of vectors in one month is related to the events in the month(s) before, the study in trimesters/seasons was also carried out to acquire more information. Also, climatic variables, NDVI amongst others, have a clearly seasonal pattern.

A devoloped explanation on the relation between each significant variables and the amount of vectors trapped was provided in the previous chapter. Considering this and the fact that the data applied for the seasonal analysis is the same of the monthly analysis, this chapter will only include a comparison with the previous section for each complex.

For the Obsoletus complex the mean and minimum temperatures had a positive effect in the warmer season (summer) in opposite with the maximum temperature in the colder season (spring), during the study period. The altitude had again a positive influence in the warmer season. This is in accordance with the scenario in the monthly analysis. Conversely, the

average of the wind speed and the NDVI had a positive influence in the spring. The NDVI, as explained earlier is important to establish breeding sites. For the Obsoletus complex they favour more densely vegetated habitats (bigger NDVI), experiencing reduced solar radiation (umbrophily).

For Pulicaris complex, similarly with what happened for the monthly analysis, the NDVI had again a negative effect (in the summer), the altitude had a positive effect in the warmer months, together with the animal density. Conversely, there is a positive relation between the maximum and minimum extremes in the temperature for the spring and a negative relation with the relative humidity.

These results agree with studies in previous outbreaks in Europe, indicating that BTD is most likely to occur during the warmer months when biting midges are more active.

6.3 Geographical distribution of Obsoletus and Pulicaris complexes in Austria in the study period

6.3.1 Monthly risk maps

The risk maps for the Obsoletus complex highlight an expected high vector presence in almost all of the Austrian territory. Starting in the North Eastern zones and stretching to the South West regions of the country. A difference between months was predicted, but the number of vectors trapped generally was expected to increase in the warmer months and to start decreasing in September. According with previous studies (Sibermayr, 2009), the South Eastern Alpine Foreland of Austria experiences the highest vector concentration influenced by mild climate conditions through Mediterranean high-pressure systems. This preference reflect the fact that *C. obsoletus* is a Northern Palaearctic species. It also probably adapted to cold, requiring relatively low temperatures for optimal development and survival. For the Pulicaris complex, the number of vectors predicted was much lower compared with the Obsoletus complex but should not be neglected. The dominance of the Obsoletus complex among the other vectors is in accordance with the situation in the other European countries. Pulicaris complex, even in smaller amounts are predicted to be widespread in the country in opposite to what was expected according with previous studies (Savini et al., 2003; Mehlhorn et al., 2007; Racloz et al., 2007 and Meiswinkel et al., 2008a). The expected spreading pattern was similar in the way that they start to spread from the Eastern regions of the country to the West. A peak in the amount of vectors trapped in the Southern areas was predicted in August, corresponding with the second peak in the entomological program data. In September it was also expected a decrease in the number of vectors trapped.

6.3.2 **Season risk maps**

In the risk maps for the Obsoletus complex it was expected an increase in the vectors number and distribution from the East to the West, with a possible peak in the summer, especially in the Southern areas.

For Pulicaris complex the number of vectors predicted was much lower in the spring (less than 100). Conversely, in the summer, only a small area in the country is expected to have large amounts of vectors trapped but not has massive has Obsoletus complex.

6.4 **Problems with the data**

When produced maps don't match with what is expected, problems may have arised from the beginning of the process, during the surveillance project excursion and idea, through the manipulation of the data, up to the end, in the construction of the risk maps. When using database recordings, the history behind the many different variables in the database used must be known as this has implications for judging the data quality and for the interpretation done. Each of the data sources has possible sources of errors that can interfere with the results, and thus, misleading the conclusions.

Starting with the entomological surveillance, the traps were mounted in heterogeneous positions, concerning exposure to sun and wind, as well as vicinity to indoor stable areas. Additionally, the following of the protocol of the traps wasn´t monitored in this study. Lack of uniformity difficult the statistical analysis of possible relationships between phenology and environmental situations. According with previous works from EFSA (2007c), it is clear that light trap results can be biased because the catches do not provide reliable information on host biting rates or feeding preferences, since non-engorged nulliparous and parous females are predominantly captured and at unknown rates compared with blood engorged females. Bias may also have been introduced into data by trapper behavior (McCarthy, 2008).

Although light trap data are essential to elucidate the epidemiology of BTD and to indicate areas at risk, their acquisition depends upon the collection of *Culicoides* in the field. The larger the survey the more important it is that the methods (and instruments) of capture be standardised to ensure that all data are comparable and informative at many levels.

In this work, the weather information came from ground weather stations. This have ready biological significance, but are recorded synoptically at a relatively small number of weather stations that are often distant from trap sites, and between which it is necessary to interpolate, to obtain a continuous layer of climatic information. When Baylis and his colleagues (2004)

were modelling the distribution of BTD vectors they concluded that suitably processed imagery is generally a better predictor of *Culicoides* than are ground-collected climate data.

In the execution of the statistical modelling, whenever a linear regression model is fit to a group of data, the range of the data should be carefully observed. Attempting to use a regression equation to predict values outside of this range, extrapolation, is often inappropriate and may yield non credible answers.

Risk maps can only be as good as the data from which it derives, whilst the prediction can only be extended to other similar geographical region of risk. The techiques cannot identify new areas of risk in completely diferent geographical settings (Staubach et al., 2008).

6.5 **Complexity of BTD**

BTD is transmitted by more than one vector species which complicates the disease epidemiology and also invalidates (partially) risk models for each vector (Meiswinkel et al., 2004). Nevertheless, models are usually based on data from one *Culicoides* species, and extrapolation to other species may not be appropriate (Tabachnick, 2004).

In Austria, the most important BTD vectors exist as species complexes (such as Obsoletus and Pulicaris complexes) and the members of these complexes may occur simpatrycally or allopatrically which makes their distribution and abundance data difficult to interpret. Since individual members may differ widely in vectorial capacity, it is vital that they be distinguished to understand exactly which species, within a complex, plays a vectorial role. Consequently, the risk of transmission may vary dramatically at the national level, depending upon the particular cryptic species present in a given area (Baldet & Delécolle, 2006). Zones of overlap will serve to facilitate the further movement (and maintenance) of the virus, and so, complicates the development of rational livestock movement and disease control strategies.

To obtain data that are reliable, modellers depend heavily upon an accurate taxonomy as each vector species, in occupying a specific niche in nature, possesses a unique biology that determines its distribution in both space and time (Meiswinkel, Labuschagne, Baylis & Mellor, 2004). Another importante issue is that vectors of BTV elsewhere in the world, are known to transmit also other viral pathogens that can be circulating also if introduced into Northern Europe during climatically favourable periods (Meiswinkel et al., 2008a).

7 CONCLUSION

From the analysis of the risk maps, it's expected that the distribution pattern and the variation in the abundance are similar, when comparing month and season analysis in both Obsoletus and Pulicaris complexes. Also, both complexes start to spread from the Eastern regions of the Austrian territory to the West, but the relative abundance of Pulicaris complex is much lower. Nevertheless, both complexes in study are expected to increase in the warmer months and to decrease in the colder ones.

The widespread occurrence and abundance of Obsoletus, place a significant portion of Austria at risk. According with the entomological surveillance, members of the Pulicaris complex, occurs in a few sites and in small amounts in Austria. Therefore, in terms of environmental conditions, only a narrow portion of the potential niche of this species was represented in the models resulting in a portion of predicted potential distribution and abundance areas. The large differences in the predicted amount of the different vectors have important implications for disease surveillance and control. Both were predicted to be present almost continuously across Austria. If Pulicaris complex midges are also widely dispersed as the model predictions indicate, this further increases the area of Austria at risk of BTD.

Presence of the appropriate breeding habitat is central to the issue of vector prevalence. This is because nourishment and development of the larval stages remains key to the successful production and persistence of viable adult *Culicoides* population in the field. Nevertheless, each vector has its own preferences which increase the possible breeding habitats available. Also, the climatic determinants of distribution are species specific in Austria. This was indicated by the different rank and order of climatic variables added to the model. Nevertheless, unstable climatic events may be capable of transforming stabile system into one that is potentially chaotic, and therefore unpredictable. Consequently, it is possible that the distribution of BTD may change (Ward & Carpenter, 1995b). Rapid changes in climatic conditions can affect vector population structure and alter transmission rates of disease (Racloz et al., 2008). While climate change may be in part driving the spread of BTD it is important to consider that there may be other contributing factors, as yet unidentified. Further field research is needed to deeply identify and understand the influence of these factors on vectors ecology and if possible, the way to use them to control the vectors.

The results of this model can be used as input parameters for the creation of a national targeted surveillance program, tailored to both the spatial and the temporal aspect of the disease and its vectors. In this manner, financial and logistic resources can be used in an

optimal way through seasonally and geographically adjusted surveillance efforts. This model can also serve as a toll for other vector borne diseases including human zoonotic vectors which are likely to spread in Europe (Racloz et al., 2008).

This work also encourages the use of advanced tools, such as GIS and statistical modelling, in veterinary medicine to prevent disease outbreak. This way, professional would only act in terms of the prevention sphere avoiding all the losses and costs with disease control.

7.1 **Suggestions for the future**

All the intervenients in the surveillance programmes should be aware of the final purposes and understand their role in the overall process so that they can cooperate in the best way. In this way, it is essential to conduct these programmes simply and in a standardized way, both in the field and in the laboratory. These aims can be achieved only with data that are both comparable and sensitive. Targeted surveillance system, could provide the basis for creating a surveillance system directed to higher BT-risk regions in specific months, for instance, increasing serological testing or avoiding trade of animals during a specific period or in a specific geographical area.

It is imperative to identify taxa accurately, to follow-up changes of populations of species and not complexes, and the determination of the virus titre of parous midges, so that field data can be interpreted correctly (Meiswinkel et al., 2008d). Identification of all competent vector species and populations coupled to an improved understanding of the relationship between temperature and virus transmission by *Culicoides* is also imperative in this process. This will allow more effective targeting of control measures against BTD vectors, the virus spread and its maintenance in the field (Paweska, Venter & Mellor, 2002). As long as the taxonomy, and the precise ecologies, of vector *Culicoides* in Europe remains unclear, it is expected to make only limited progress in terms of predictive risk modelling and in the development of national control strategies (Meiswinkel et al., 2004b). However, molecular techniques are now being developed and can, therefore, enable accurate identification by non-morphological specialist.

The proportion of a midge population involved in daytime attacks is not known. It is not possible to monitor diurnal *Culicoides* activity using light traps only. For this, different capture methodologies are needed (e.g. bait animals, suction traps, CO_2 traps and truck traps) (Meiswinkel et al., 2008). Since *Culicoides* spp. is strongly dependent on micro-climatic conditions it might be advisable to adjust the trapping design and include different daytimes and locations outside the immediate range of ruminants.

Further analysis of entomological, meteorological and agricultural datasets and laboratory work is required to identify possible routes, together with improvements in the ability to predict and plan for the appearance of BTV. Mathematical modelling and remotely sensed data enable the identification of areas and periods at risk from introduction to be identified (Purse et al., 2004a; Gubbins, Carpenter, Baylis, Wood & Mellor, 2008) and likely patterns of spread to be predicted (Wilson & Mellor, 2008).

Even though no specimens of *C. imicola* were identified in the course of this study they are expanding to Northern regions due to the effects of global warming (Purse et al., 2007). *Culicoides* spp. is known to transmit other diseases which could potentially be introduced into central Europe in the near future (Meiswinkel et al., 2008a; Wilson & Mellor, 2008). Therefore, risk assessments might be useful for other emerging vector-borne diseases which are about to advance to Central Europe. The development of high resolution spatial models of vectors distribution improves the forecast of the possible spread of this and other arboviruses diseases, after a detected outbreak (EFSA, 2007a). It also may highlight areas that are vector free and thus suitable for the quarantine of livestock, to facilitate the development of a safe disease free export trade in ruminants.

Foci of risk should be pinpointed because they could be man made and thus amendable to control.

BTD can also affect wildlife and hold a great challenge for corrective measures in case of a disease outbreak. Susceptible wild ruminants should be included in surveillance programs when these animals are intended for trade. This could dramatically minimize economic losses by early detection and rapid response (Meiswinkel et al., 2008a).

The amount of knowledge about a population is called realism and increasing it generally demands increasing model complexity (Ward & Carpenter, 1995a). Sometimes the ability to make precise quantitative predictions in specific time and place might be far from what we expected in the beginning of the modelling process. It depends on the complexity of disease epidemiology, financial and human resources, amongst other factors. Nevertheless, modelling can still make important contributions (Caswell, 1998).

The results of this study can help in surveillance programmes in the design of a targeted surveillance program, for optimal utilization of human and financial resources for instance in the time period of risk for BTD outbreaks.

GIS mapping software in combination with spatial analysis and statistical modelling as predictive tools, have been used in other countries and stress the advantages of using different technological methods in supporting surveillance efforts; as well as for other purposes aiding

in the improvement of health and prevention in both public and veterinary terms. An advantage of GIS methods includes the ability to improve prediction maps, once more comprehensive field data has been collected, and adjust surveillance efforts in a timely and accurate manner.

Flexible surveillance programs should be used in order to attribute financial and human resources to high-risk areas considering temporal and spatial factors (Racloz et al., 2008). GIS use in veterinary medicine is evolving to become far more than an integral part in decision support systems for the control of animal diseases. The creation of a disease early warning system based on a real time disease surveillance combined with statistics and GIS, would be extremely important in the overall process of prevention and control of BTD and other diseases with global impact (Purse & Rogers, 2008).

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